TAN TAO UNIVERSITY
FROM KNOWLEDGE TO THE STARS

Final Examination
Subject: Nanobiotechnology applied in medicine (AB304)
Time: 90min

Name:_____

Student's number:_____

Questions:

1. Describe the consequences of Nanoparticle-Mediated Complement Activation and Fixation. (30p)
2. Describe targeting, incorporate of drugs into nanocarriers, and choosing carriers in targeting Cancer Using Nanocarriers topic. (40p)
3. Describe nanotechnology Strategies in the Development of Drug Releasing Implants. (30p)

Answers:

1. **Describe the consequences of Nanoparticle-Mediated Complement Activation and Fixation**.

Opsonisation and Phagocytic Elimination

Complement opsonisation and subsequent macrophage recognition of particulate drug carriers offers an unprecedented opportunity for intravenous delivery of therapeutic agents and immunomodulators to phagocytic cells in the liver (Kupffer cells) and the spleen (marginal zone and the red-pulp macrophages).

Nanoparticle Integrity in the Blood

Complement activation can significantly affect the integrity of certain classes of drug carriers in the blood. For example, insertion of MAC into the liposomal bilayer may lead to substantial leakage of entrapped (aqueous) cargo, but this may be minimized or prevented through surface PEGylation.

Adjuvanticity

Nanoparticles have been used as immune potentiators or adjuvants triggering elements of innate immunity that subsequently assist the generation of potent and persistent adaptive immune responses. Most of these efforts are being directed to enhance the immunogenicity of subunit vaccines through both antigen protection and targeting to antigen-presenting cells as well as immunostimulation. The role of the complement system in some of these processes is rather intriguing.

Nanoparticle-Mediated Infusion-Related Adverse Reactions

Acute allergic-like reactions with haemodynamic, respiratory, cardiovascular, cutaneous and gastrointestinal manifestations, which are not initiated or mediated by preexisting
IgE antibodies, have been reported to occur in approximately 45 % of individuals within a few minutes of infusion of nanomedicines (e.g., liposomal drugs such as Abelcet ® , Ambisome ® , DaunoXome ® , Doxil ® , Myocet ® and Visudyne ® ; micelle-solubilized drugs such as Taxol ® , Taxotere ® and Vumon ®) and diagnostic nanoparticles (e.g., dextran-coated iron oxide nanocrystals such as Feridex ® and Combidex ®). In some isolated cases this has been fatal (e.g., Taxol ®). Compelling evidence suggests that inadvertent activation of the complement system is an important factor in eliciting these reactions. This is partly due to the liberation of potent complement bioactive products (e.g., C3a, C5a and C5b-9) with the ability to modulate the function of a variety of immune cells (e.g., monocytes, polymorphonuclear cells, platelets, mast cells) and vascular endothelial cells, and partly to cross-talk with Toll-like receptors.

Nanoparticle-Mediated Tumour Growth

A recent study in immunocompetent mice, as well as in C5 and C5a receptor knockout animals bearing a syngeneic tumour, has strongly indicated that intratumoral accumulation of complement activating long-circulating nanoparticles can accelerate tumour growth through C5a liberation. Tumour growth was more significant with nanoparticles capable of directly enhancing the alternative pathway turnover of the complement system compared with nanoparticles that triggered complement predominantly through the lectin pathway.

2. Describe targeting, incorporate of drugs into nanocarriers, and choosing carriers in targeting Cancer Using Nanocarriers topic.

Targeting

There are generally two approaches for nanocarrier-mediated systemic drug targeting from the site of administration (i.e. injection):

1. Passive targeting to tumor tissues—via enhanced permeability of tumor vasculature.
2. Active targeting at the cellular level—to cancer cells without affecting normal cells.

Passive targeting to the tumor tissues relies on 'leaky' microvascular or the enhanced permeation and retention (EPR) effect, which permits selective permeation of nanoparticles into the desired tumor tissue. Active targeting to cancer cells is possible through promoting specific interactions with targeting ligands and surface receptors. An engineered nanoparticle should be able to overcome the physiological challenges of systemic circulation including tumor vascular biology and organs of mononuclear phagocytic system (MPS) whose function is to remove foreign material from the circulation.

Incorporation of Drugs into Nanocarriers

Chemotherapeutic anti-cancer drugs and their carrier may be joined via two major techniques. One way is to entrap drug molecules into delivery vehicles for controlled release of the drugs. Controlled release occurs when a natural or synthetic polymer is combined with a drug in such a way that the drug is encapsulated within the polymer system for subsequent release in a predetermined manner. The release of the active agent may be constant or cyclic over a long period, or may be triggered by the environment or other external events. In general, controlled-release polymer systems can provide drug levels in the optimum range for an extended period of time than other drug delivery methods, thus, increasing the efficacy of the drug and maximizing patient compliance. The primary consideration of drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing. Other advantages of using controlled release delivery systems can include the maintenance of drug levels within a desired range, the need for fewer administrations, higher diffusion rates of the drug into the tumor due to a continuous-controlled release of it in the tumor proximity and optimal use of the drug in question.

Another approach is to conjugate drugs covalently to the bulk of drug carriers. carrier-drug conjugation usually inhibits metabolic activity until the conjugation bond is cleaved, resulting in activation of the drug at the target site. The covalent conjugation between carriers and drugs has a number of advantages including: (1) Limiting the mechanism of drug release from the carriers and (2) reducing potential burst of drug as it is typically seen in entrapment approaches.

Choosing a Carrier

In choosing an appropriate nanocarrier construct, one should consider the following criteria:

1. A carrier should be made from a material that is biocompatible, easily functionalizable, and well characterized.

2. A nanocarrier with targeting molecules should exhibit high uptake efficiency by the target cells unless they are required to accumulate on the cell surface and act as site specific drug depots.
3. The nanocarriers should be either soluble or colloidal in aqueous phase and exhibit an extended circulating half-life to increase the likelihood of their effectiveness.
4. The nanocarriers should have a low rate of aggregation and preferably a long shelf life.

3. Describe nanotechnology Strategies in the Development of Drug Releasing Implants.

Drug Adsorption

The simplest method for incorporating drugs is to adsorb them onto the surface of the formed implant by drying or lyophilizing a solution of the drug onto the surface. In this case the release of the drug from the surface depends on the binding strength between the surface and the drug relative to the flow and content of the medium surrounding the implant. If this medium contains molecules that bind to the surface these may displace the drug. If slower release is desired, the drug may be incorporated into a drug delivery system that exhibits greater binding to the implant surface. A drug can also be covalently tethered to the surface, either permanently if the drug is only to affect adhering cells or temporally if the drug is to be released slowly to the implant surroundings.

Drug Encapsulation

If a slower release or greater drug protection is desired, the drug may be incorporated into the implant surface or the entire implant. This can be achieved by dissolving the drug in the polymer melt or solution prior to forming the implant, the drug then becomes encapsulated as the polymer matrix solidifies. Using this method requires that the drug can tolerate the solvent or temperature used in the implant formation process which may not be the case for labile biological drugs, in this case a solvent/water emulsion may be used so the drugs can be kept in a aqueous phase prior to dehydration.

Drug Encapsulation by Co-Axial Electrospinning

Drugs can be incorporated onto or into electrospun polymeric implant the same ways as a normal polymeric implant by adsorption or encapsulation. Electrospinning, however, offers an additional mode of drug incorporation known as co-axial electrospinning, this method deposits a core-shell fiber containing an internal core phase and an outer shell phase which may be chemically different. This allows the deposition of functional drugs into a drug

= compatible core phase even when harsh solvents are employed for the outer shell phase.

Drug Release from Metal and Ceramic Implants

Metal and ceramic implants can be coated with drugs by adsorption the same way as with polymeric implants, indeed dip coating metal prosthesis in solutions of antibiotics prior to implantation is a simple solution that has been employed. Unfortunately, such coated drugs are released fast. Drug encapsulation as a means to control the release is not as easy as with polymeric implants as most drugs will not survive forging or sintering temperatures or pressures. In these cases other approaches have to be tried. A solution is to coat the metal or ceramic surface with a polymeric or hydrogel layer encapsulating the drug for example using the LbL method. Achieving a uniform layer using this method is, however, not simple on three dimensional implants. A different solution is to electrospin polymeric fibers around an implant

Co-Release of Multiple Drugs

In many cases it would be beneficial to release more than one drug from an implant. In some cases it could be that different drugs accomplish different functions such as combating infection while promoting stem cell differentiation, whereas in other cases, drugs act synergistic to induce a desirzhenomenon. Each step in stem cell differentiation, for example, is typically promoted by more than one growth factor and co-delivering several growth factors is, thus, necessary to recapitulate natural development completely

Temporal Controlled Drug Release

Many implant related phenomenon are time dependent. Implant associated infections and acute inflammation take place immediately upon implantation, whereas, fibrous encapsulation occurs weeks after implantation and wear debris induced inflammation may not occur until years following implantation. The same is true in stem cell differentiation in tissue engineering where each differentiation step occurs in successions. Controlling drug release temporally is, thus, of great importance and can be achieved by choosing an appropriate delivery strategy that has suitable release kinetics for the biological function that needs to be modulated.

Spatial Restricted Drug Release

Spatial restriction by nanofunctionalization is useful in the cases where a differential response is desired in different parts of an implant. This is relevant when complex tissue composed of multiple cell types has to be grown.

A strategy is to load each phase with different drugs typically loaded in drug vehicles, these drugs can then be released in a spatially restricted manner and induce the desired local effects.