A shotgun approach to identifying immune evading excipients for biologics drugs.

Shireesh P. Apte*

Editorial

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Autoimmune diseases are usually treated with disease modifying drugs (NSAIDS, steroids, interferons), immune suppressant drugs (immunomodulators, biologics), or protein products (insulin) of the cells that are aberrantly targeted by the immune system. Efforts to implant or introduce insulin producing cells into the body have so far met with limited success due to immune attack. Formulators are working with several biomaterials that can be used to encapsulate islet cells in a porous matrix that can allow the cells to access oxygen and nutrients, prevent immune recognition and subsequent attack and allow their protein products (insulin) to egress the matrix into the blood.

Since the immune system response to any biomaterial excipient encapsulating live cells can only be evaluated *in vivo*, this necessitates the availability of a large number of animal test subjects to screen every possible formulation. Due to the cost and time associated with this scenario, only a limited number of formulations that show promise *in vitro* and which are heuristically deemed to be optimal are currently tested in animal subjects. Consequently, there is no guarantee that the most optimal formulation is being tested. It is no surprise therefore, that encapsulated live cell formulations in preclinical or clinical trials still require co-administration of immune suppressant drugs, which mitigates their advantage in providing long-term disease control.

An obvious solution to this problem is if these hundreds of formulations could each be tested concurrently at one time in one animal subject. If different formulations encapsulated cells with different DNA, all those formulations could be injected in one animal test subject. Information on which DNA is paired with which biomaterial or formulation would be known initially for every formulation. Screening could be achieved by sequencing the DNA of those cells found to evade immune attack, thus enabling the most effective formulation to be chosen. Just such an approach was recently reported where different alginate formulations each encapsulated human umbilical vein endothelial cells (HUVEC) from unique different donors (1).

A plethora of possibilities can be constructed. Instead of using HUVEC cells from different donors, different DNA or RNA sequence constructs, covalently bonded to the same strong immunogenic molecule (such as a suitable peptide sequence, bacterial lipopolysaccharide or immune adjuvant)

*Corresponding address: 5204 Coventry Court, Colleyville, TX 76034,
E-mail: shireeshpapte@msn.com

can be packaged into different alginate (or other matrix or colloidal structure forming biocompatible excipients) formulations. Each formulation will encapsulate a different and defined DNA or RNA sequence construct connected to the same immunogenic molecule. In this case, the formulation with the most intact DNA or RNA represents the one with the best immune evasion properties.

The excipient industry; in conjunction with CROs, can take over this initial proof of concept formulation platform. The output of such a platform would be those precisely and suitably engineered or modified excipient molecules which; when formulated into colloidal delivery systems (such as nanoparticles, microcapsules, liposomes; among others), would prevent – to various degrees - immune or antibody attack, prevent complement activation, allow nutrient and oxygen diffusivity and allow drug or protein release. These molecules could then be marketed as value-added different grades of (say trademarked) EVADE® (Escape Validated Anti-immune Detection Excipients) materials – reminiscent of; but significantly more complex than their *in vitro co-processed excipient* counterparts - depending on which attribute is the most important for a particular disease. EVADE® Alginates class I through EVADE® Alginates class III could potentially decrease the time to the clinic for formulations encapsulating live cells.

This value-addition represents the latest in the saga of how excipient molecules for biologic drugs have long begun to be designed with the primary function of modulating *in vivo* drug properties. The excipient 'bar-coding' iteration described in this editorial takes this approach even further. Testing hundreds of alginate modifications or combinations with different colloid forming excipients enables a 'brute force' approach toward *in vivo* formulation evaluation and development that has hitherto not been possible. Immune evading excipients that constitute injectable or implantable formulations containing live cells can now be identified with algorithmic precision and a near certainty of success. There is no reason to assume why this shotgun approach cannot be extended to identifying colloid forming excipient constituents that target a particular organ, cellular organelle, signalling pathway, or to the deliberate elicitation of a desired immune response. It seems to the author that excipient choice and/or design for biologics may well shift from being the quintessential formulator's *art* into an algorithmically compressible realm. After all, Deep Blue did defeat Garry Kasparov.

REFERENCES

1 Sudip Mukherjee *et.al.*, Screening hydrogels for antifibrotic properties by implanting cellularly barcoded alginates in mice and a non-human primate, https://doi.org/10.1038/s41551-023-01016-2