Nanomedicine: new hope for transplant paradigms lost?

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“Transplant nanomedicine can help us accomplish what transplant immunosuppression has failed: namely precisely targeted and personalized treatment with minimal or no collateral systemic side effects.”

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“The supreme art of war is to subdue the enemy without fighting: be extremely subtle even to the point of formlessness. Be extremely mysterious even to the point of soundlessness. Thereby you can be the director of the opponent’s fate.” – Sun Tzu, The Art of War.

Nanotechnology and nanomaterials (such as nanoparticles, nanocrystals, nanocomposites and nanoelectronics), unbeknownst to many, have emerged as an ubiquitous presence in our daily lives. Nanomedicine refers to the application of nanotechnology to medicine [1]. Imaging tools or technologies and treatment interventions tailored at the nanoscale level (one-billionth of a meter) have potential for game-changing impact in precise diagnosis and prognosis, preventative management and personalized therapies.

There has been tremendous interest, investment and progress in cancer nanomedicine over the past two decades [2]. Nanomedicine approaches have facilitated safer, more effective and accurate tumor targeting at very low doses of drugs, prolonged half-life/activity and opened possibilities for combining diagnosis and therapy in one agent [3]. Such ‘theranostic’ nanomedicine could help personalize chemotherapeutic delivery, monitor as well as optimize treatment adequacy or efficacy and minimize toxicity to improve cancer survivability.

In contrast to cancer nanomedicine, the field of transplant nanomedicine is still in its infancy [4]. A PubMed search of the keywords ‘nanomedicine in cancer’ for studies over the past decade results in around 10,000 hits (including many clinical trials) while the keywords ‘nanomedicine in transplantation’ over the same period results in around 800 hits (mostly reviews and no clinical trials). Testament to this is the fact that transplant nanomedicine continues to feature poorly in the scientific agendas of the largest national and international transplant congresses.

The concepts, techniques and accomplishments of two Nobel laureates, Alexis Carrell [5] and Joseph Murray [6], changed the face of reconstructive surgery and organ transplantation as we know them today. The advent of vascularized composite allotransplantation (VCA) over the past two decades heralds a new era for reconstructive surgery. VCA overcomes the limitations of conventional reconstructive procedures by eliminating donor site morbidity or the need for autologous tissues. Over 300 patients worldwide have benefited from the life-changing or life-giving benefits of VCA procedures in conditions lacking options for conventional reconstruction. Yet, VCA is fraught with hurdles including dependence on chronic immunosuppression, limited donor availability due to external matching requirements and debatable exit strategies in the event of graft failure [7].

The past decade has witnessed the convergence of reconstructive surgery with tissue engineering and regenerative medicine leading to the inception of the new field of regenerative surgery. The driving force for such multidisciplinary integration is to overcome the current limitations of vascularized organ or tissue transplantation. Nanomedicine is a timely, groundbreaking means to that end.

This special issue of *Nanomedicine*, titled ‘Nanomedicine in Organ Transplantation and Regenerative Surgery – Advances, Applications and Future Directions’, is focused on the interface of nanomedicine with organ transplantation and regenerative surgery.

In this issue, contemporary, state of the art commentaries and reviews exclusively address nanomedicine applications relevant to therapeutic, diagnostic and monitoring strategies in solid organ transplantation and regenerative medicine/surgery. Essential aspects of nanotoxicology, biocompatibility and nanopharmaceutics are also covered, spanning topics such as design, manufacturing, biosensing and therapeutic drug delivery.

In the next two decades, we will witness major progress in nanotechnology applications in plastic and reconstructive surgery as well as in VCA. These include but are not limited to: nanosensors (for monitoring transplant or free flap outcomes and efficacy of therapeutic interventions); nanocarriers for delivery of oxygen (for tissue preservation) or nanoscale biologics/drugs (for treatment of rejection or improving nerve regeneration) or nanoimaging of neuroregeneration (for improving functional outcomes after replantation or VCA). Wong *et al.* make the case for such progress in reconstructive surgery through applications of tissue engineering and regenerative medicine facilitated by nanotechnology [8]. They predict there will be a big impact in the area of wound healing (e.g., biodegradable drug eluting dressings, microbiological or oxygen sensors), nerve regeneration (e.g., molecular imaging), soft tissue or bone reconstruction (e.g., prefabricated vascularized conduits or bioscaffolds for stem cell or fat graft delivery, smart nanocoatings for breast implants, 3D printed biointegrable nanocomposites for bone regeneration), *ex vivo* engineered tissue interfaces and vascularized composite constructs, and targeted nanomedicine strategies for multipronged applications (e.g., antirejection, antifibrotic or antineoplastic therapies to improve outcomes of conventional reconstructive or VCA procedures) [9].

Static cold storage (at 4°C) with University of Wisconsin (UW) solution remains the current standard of care for tissue preservation in solid organ and VCA due to ease, simplicity and logistical advantages [10]. Since it lacks an oxygenated perfusate, static cold storage leads to tissue hypoxia with compromise of cell membrane integrity, tissue edema, acidosis, shut down of cellular respiration and cell death. Whole blood is a suboptimal alternative because of shelf lability, supply shortages and infectious risks. Second generation acellular hemoglobin oxygen carriers (HBOCs) have the advantage of availability, compatibility and superior stability compared with whole blood. However, the oxygenation benefits of HBOCs come at the expense of capillary vasoconstriction due to endothelial nitric oxide scavenging during ischemia, and activation of complement, kinins and platelets during ischemia reperfusion injury, which has a major impact on acute and chronic outcomes after VCA or organ preservation.

Lambert *et al.* provide a highly comprehensive review of the unique properties of perfluorocarbons (PFCs) as safe yet versatile nanomedicines with multifunctional properties such as drug/oxygen delivery, multimodal imaging capability, retention and targeting characteristics [11]. They propose PFC nanoemulsions as unique oxygen-carriers that could represent a paradigm shift in cell, tissue or organ preservation. Such nanotechnology is a significant advance beyond existing perfusates (such as packed red blood cells [RBCs], second generation HBOCs or UW). It is chemically and metabolically inert (not bovine or human derived), nonimmunogenic and nonvasogenic (does not cause vasoconstriction) and provides effective intracellular oxygen delivery to mitigate the effects of ischemia reperfusion injury and its subsequent deleterious immune or neuronal effects in transplants. Also, unlike other HBOCs and blood products (purged RBCs) used for oxygen delivery, PFC-nanoemulsions manufactured using standardized, quality by design approaches, are remarkably shelf stable for prolonged periods (>300 days) even at 37°C and have no impact on immune cell activation.

Currently, transplant immunosuppressive drugs are delivered orally (enteral administration) in solid organ transplants and VCA [12]. These agents like tacrolimus (Prograf®), mycophenolic acid mofetil (Cellcept®) or rapamycin (Rapamune®) undergo first-pass metabolism in the liver, greatly reducing their bioavailability due to actions of enzymes of the gastrointestinal lumen and lining, bacterial enzymes and hepatic enzymes. Combined with possible renal clearance, only a small percentage of the drug typically reaches target graft tissues, resulting in very high ratio of systemic versus local graft exposure. Consequently, large and repeated dosing is often necessary, which can increase the risk of toxicity. Covvey *et al.* overview pharmaceutical care in transplantation. They address limitations of current transplant drugs in terms of end organ toxicity and therapeutic efficacy and highlight novel nanomedicine formulations with promise for clinical application [13].

For optimum therapeutic effect, the right amount of a drug needs to get to the right place at the right time. In transplantation, therapeutic efficacy is directly driven by the delivery and tissue targeting of the drug. Nanomedicine-based drug delivery formulations must be designed so that they do not simply release a drug at a specific rate, but

release the drug in ways to improve safety, efficacy, convenience and patient compliance. In their paper, Vemula and colleagues examine drug delivery in organ transplantation and regenerative surgery applications. Topical or implantable, sustained release, prodrug-based and stimulus-responsive nanoscale biomaterial based drug delivery systems are discussed along with the benefits of nano-formulated transplant immunosuppression [14]. First, graft-embedded nanoformulations provide strategic and precise spatial control of a bioactive drug depot in transplant tissues. Such localization greatly reduces the required dose, and often the systemic toxicity. Second, nanocarriers can enable temporally constrained release, sustaining therapeutic concentrations over a longer time period as compared with oral or parenteral delivery. Third, in addition to immunosuppressive drugs, nanoparticles can also be used to locally release biologics, growth factors or other agents sensitive to tissue degradation or with short half-lives. These strategies could be relevant in improving neuroregeneration or prevention of graft vasculopathy due to chronic rejection. Despite these advantages and potential for significant impact on both patients and outcomes, nanomedicine based approaches have not been seriously explored in organ transplantation or VCA [15].

Current state of the art techniques such as mass spectroscopy for measurement of immunosuppressive drug levels in transplant recipients require intravenous blood draws and access to specialized laboratories. Inadequate systemic concentrations of immunosuppressive drugs can lead to tissue/organ rejection and high concentrations can lead to toxicity. Moreover, the systemic blood level of immunosuppressive drugs may not accurately correlate with local graft tissue levels [15]. This is compounded by the fact that these drugs have high inter-individual pharmacokinetic and pharmacogenetic variability and food and drug interactions. Thus, frequent, precise and point of care monitoring of immunosuppressive drug concentrations is important to inform timely and tailored treatment decisions in transplant patients. In their paper, Shafiee *et al.* review novel advances in nanobiosensors for point-of-care, rapid therapeutic drug monitoring from a minute amount of blood (100 µl or less). The thresholds, range and rapidity of detection, reliability and reproducibility, point of care capability, sensitivity, specificity and positive predictive correlation with gold standard assays are key considerations in development of nanosensors for transplant applications [16]. Multiplexing of such sensors into a rapid, inexpensive, ultrasensitive, diagnostic platform could enable femtomolar range sensitivities for a range of analytes of interest, including transplant rejection biomarkers reflective of antigen-specific humoral and cellular immune responses [17].

Peripheral nerve injuries are commonly seen in reconstructive surgery practice and are common in civilians (motor vehicle accidents, lacerations with sharp objects, penetrating trauma, stretching or crushing trauma and fractures, and gunshot wounds), as well as in war injuries (due to blast trauma) [18]. Unlike in solid organ transplants, the need for nerve regeneration is a key demand, unique to VCA. Although functional outcomes after clinical VCA such as upper extremity or face have been acceptable in some patients, in many cases, sensorimotor recovery has been suboptimal or poor. Such inadequate or ineffective nerve regeneration can result in loss of graft function even in an immunologically viable graft [19]. Thus, the ability to objectively diagnose nerve injury or monitor nerve regeneration in transplant patients in a sequential or real time, non-invasive manner is a significant step in the management of these patients [18].

Current challenges lie in accurately monitoring regeneration in patients because surgical biopsy of nerves is not feasible. State of the art techniques, such as electromyography and nerve conduction studies, have several limitations, including poor localization. MRI is capable of imaging nerves with high spatial resolution. Nanoimaging with immune cell targeted MRI agents [20–22] can be adapted for monitoring immune system dynamics following nerve injury and in VCA [23]. MRI can also be combined with near-infrared fluorescence imaging of neuroinflammation and correlated with functional behavior [24,25]. Though useful in preclinical studies, MRI presents with limitations. It is expensive and not portable making it nonideal for longitudinal studies of nerve growth in the clinic. Furthermore, MRI is not safe for patients with embedded metal shrapnel or medical devices. Ultrasound on the other hand is inexpensive, portable and noninvasive, but only lacks 3D spatial, anatomical resolution. In their paper, Berezin *et al.* discuss these and other imaging modalities with specific emphasis on noninvasive nanoimaging modalities that could help patients undergoing regenerative, repair or transplant strategies for nerve injuries [26]. Novel neurotherapeutic nanoagents are currently in development with the aim of enhancing nerve regeneration via different mechanisms and distinct biochemical pathways [24]. The ability to accurately monitor nerve regeneration across the growth cone or assess surrogates of degeneration (myelin debris and axonal edema) could open new avenues of research into effective drugs and therapies. Nanoimaging based monitoring of nerve regeneration would allow us to better personalize treatments as well as determine safety and efficacy of such therapies.

Important goals of nanomedicine are to reduce toxicity of drug dosing and limit systemic exposure through improved retention and targeted activity. However, the nanoscale platforms themselves can contribute to *in vivo*

toxicity. The smaller the size of the nanoparticles or nanoscale agents, the greater their surface area-to-volume ratio and the higher their chemical reactivity and biological activity. Notably, the extremely small size of nanomaterials used in drug delivery could help them migrate across physiologic barriers (such as the blood–brain barrier), or be retained for unknown duration in the body following phagocytosis by macrophages [27]. Such retention may have unknown long term consequences including but not limited to inflammation, oxidative stress, cytotoxicity, genotoxicity or biocompatibility issues. Toxic effects could vary depending on composition, size and route of administration. Policy and regulatory concerns for toxicology of nanomedicine are paramount considerations during formulation and development. This is the driver for renewed focus on quality by design and standardized practices in nanomanufacturing during scale up and commercialization of nanomedicine.

In their paper, Mallory *et al.* detail applications, considerations and hurdles of nanotoxicology as relevant to transplant drugs/nanomaterials [28]. The drivers of toxicity, such as intrinsic elemental characteristics, morphology, surface reactivity of nanoscale materials and effects of unbound versus bound nanoscale materials and variables (such as size, shape, solubility, porosity and surface elements) that influence toxicity are delineated. Importantly, *in vivo* toxicity considerations of novel transplant drug delivery platforms are examined with reference to multi-compartmental or physiology based pharmacokinetic modeling in conjunction with *in vitro* models of toxicity. In their article, Zor *et al.* address the issues of biocompatibility of nanoformulations and their interactions with host immune cells and tissue environment [29]. Such ‘immunocompatibility’ may be an important determinant in the therapeutic efficacy of nanomaterials, as they can stimulate or suppress the immune response based on factors such as dose, route of delivery, duration of exposure, fate, biodistribution, translocation or elimination.

Lessons learned from cancer nanomedicine and nanotechnology applications in other areas, will help guide rational, biomimetic and biocompatible platforms, quality by design formulations and standardize scale up manufacturing processes. Active drug targeting (through intracellular or surface receptor uptake) or passive drug targeting (through enhanced permeability and retention), can help effective, selective and specific targeting of nanomedicine (antibodies, peptides, small molecules and drugs) while improving safety and toxicology profiles and maximizing efficacy. Sophisticated, multifunctional or multimodal theranostic nanomedicine allows delivery of therapeutic and imaging agent payloads in sustained or stimulus responsive delivery platforms to simultaneously treat disease and monitor disease response, remission or relapse. Transplant nanomedicine can help us accomplish what transplant immunosuppression has failed: namely precisely targeted and personalized treatment with minimal or no collateral systemic side effects. In summary, we are poised for a major watershed moment for implementation of nanomedicine in transplantation and regenerative surgery indications.

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