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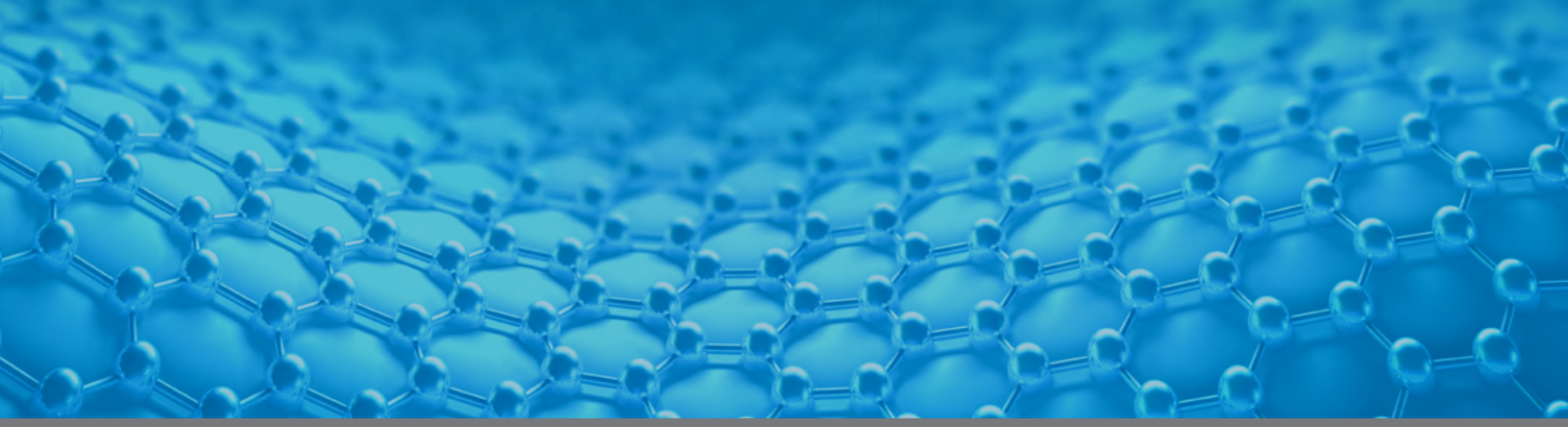
NANOTECHNOLOGY'S POTENTIAL TO TRANSFORM ORGAN TRANSPLANTATION

Authored by

Jedediah Lewis, Dr. Alyssa Ward, Dr. Greg Fahy, Mark Severs, Dr. Boris Schmalz, and Dr. Gloria Elliott

on behalf of the Organ Preservation Alliance



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The growing organ shortage costs tens of thousands of lives and billions of dollars in healthcare expenditures each year, according to a recent roadmap from the Organ Preservation Alliance.[1] In previous efforts, nanotechnology has been identified as a key platform that can lead to advances in organ banking and transplantation.[2,3] **Nanotechnology is poised to play a pivotal role in organ preservation and transplantation** in the coming years.

A consensus article in *Nature Biotechnology* discussed the need for engagement of researchers from various disciplines, including nanotechnology, to coalesce and utilize new and emerging technologies to make organ banking a reality.[4] This was solidified at the ***Summit on Organ Banking through Converging Technologies***, which explored the potential of nanotechnology to facilitate organ and complex tissue banking. More broadly, nanotechnology has been identified as an enabler of advances in transplantation.

Focused federal support for nanotechnology research in the last decade has led to major advances imaging,[5,6] biosensing,[7,8] bioagent delivery,[9] and energy transfer[10] – each of which can play important roles in organ preservation. Two high-impact areas that are well positioned for innovation are discussed.

TRANSPLANT ORGAN ASSESSMENT

One of the major drivers of the organ shortage is the limited ability to assess whether a donor organ is suitable for transplantation. Because preservation times are currently limited to hours, organs must be transplanted immediately after procurement and are conventionally stored under hypothermic (4°C) conditions.[11] This has historically provided little opportunity to assess organ health and function, critical to determining whether the organ is suitable for transplantation.

This organ assessment gap contributes to the **inability to transplant thousands of organs each year in the U.S.**, as transplant centers must routinely turn down organs that are likely transplantable in large part due to limited information about their health and function.[12,13] Today almost 70% of donor hearts and over 80% of donor lungs remain unused.[14,15] While a single donor can provide up to 8 lifesaving vital organs, on average only 2-3 are actually transplanted.[15]

Over the last decade, **breakthroughs in organ preservation have laid a foundation for a revolution in organ assessment capabilities,** which could allow many of these "borderline" organs to be transplanted by removing uncertainty about their suitability for transplantation. Organ perfusion platforms that mimic the organ's physiological environment are beginning to come into clinical use, providing opportunities to evaluate new aspects of organ health and function before transplantation. As these platforms advance, the field of transplantation will require assessment methods that are highly sensitive and give information in real time.

Nanotechnology is ideally suited for these challenges. Nanoparticles are used for contrast enhancement and signal amplification in a large and growing range of applications, including 3D imaging of biomarkers and rapid pathogen detection. Nanotechnology-based imaging and rapid, ultrasensitive detection methods have the potential to become platform technologies in organ assessment.

Because nanoparticles can be decorated with recognition sites for a virtually unlimited array of markers, they can be adapted to assess multiple indicators of suitability for transplantation, varying by organ. This flexibility can also drive advances in transplant organ assessment, allowing ever more useful clinical markers to be discovered.

Nanotechnology-based strategies that could enable breakthroughs in organ assessment include:

Assessment of tissue viability and stress

Nanosensors have been used to detect biomarkers for cellular stress and death even at low levels,[3,16–18] enabling 3-dimensional imaging to assess tissue health.[16,17] This approach has been used experimentally to detect tissue damage from ischemia, the primary source of injury to organs during transplantation, in animal hearts both before and after removal from the body.[17]

Assessment of tissue inflammatory state

Both donor death and ischemia from the transplantation process cause significant inflammation, impacting the likelihood that a transplant will be successful.[19] Nanoparticles have been used to detect inflammatory markers on blood vessel walls.[20] This offers an appealing strategy for organ assessment, as the nanoparticles do not need to permeate tissue so assessment can be performed more rapidly.

Assessment of tissue metabolic state

Significant metabolic changes in some organs have been associated with the degree of injury during transplantation, making metabolic markers such as adenosine triphosphate (ATP) levels valuable predictors of suitability for transplantation.[21] While traditional detection methods have suffered from limited sensitivity, nanoparticle-based methods such as surface plasmon resonance (SPR) can detect levels of ATP or other metabolic biomarkers with high sensitivity.[22] Using microfluidic platforms, SPR detection can be made rapid and inexpensive,[8] ideal for evaluation of organ metabolic state during short time windows before transplantation.

Detection of transmissible diseases

While rates of cancer and infectious disease transmission through organ transplantation are estimated to be below 1%, they remain on the order of 10,000 times higher than in blood transfusion, where dramatically longer shelf life (weeks, rather than hours) enables time-consuming pathogen detection methods.[23] High-profile cases of disease transmission also affect public perceptions of transplant safety, of great concern to the transplant community.[24] A variety of nanoscale diagnostic devices have made rapid, highly sensitive detection of transmissible diseases practical and are now being developed for early cancer detection and point-of-care.[8,25,26] The cost-effectiveness and speed of detection (often minutes) make them ideal for disease screening in organ transplantation.

ORGAN AND COMPLEX TISSUE BANKING

The ability to bank organs and complex tissues through cryopreservation would alleviate a large breadth of logistical constraints in both organ transplantation and tissue engineering, greatly increasing access to organ replacement and potentially **saving millions of lives in the coming decades.**[27,11,28] An NSF-sponsored technology roadmapping process identified nanotechnology as one of the major emerging fields that remains largely untapped in organ banking research.[27]

Organ banking challenges are uniquely amenable to nanotechnology solutions. Whereas cryopreservation of cells has been achievable for decades using relatively simple protocols,[11] cryopreservation of large tissue systems is a much more complex proposition. Organ banking would benefit tremendously from precise spatial and temporal control of multiple processes, as it entails:

- Competing needs for solution viscosity, which should be low during some parts of the cryopreservation protocol and high during others.
- Mechanical stresses caused by uneven heat transfer within the organ, due to its size and tissue heterogeneity. This can cause tissues to fracture.
- Addition of cryoprotectants that are toxic at high temperatures but protective at low temperatures.

Nanotechnology allows us to address each of these challenges, both by providing unprecedented control of the timing and location of key chemical and physical phenomena and by enabling real-time imaging to guide the cryopreservation process.

Nanotechnology-based strategies that could enable breakthroughs in organ banking include:

Nanowarming

To address the challenge of mechanical stress caused by uneven heat transfer, an award-winning approach to cryopreservation uses iron oxide nanoparticles in combination with low-frequency radio waves.[2,29,30] Tissues are perfused with nanoparticles before cryopreservation then excited with radio waves during rewarming, causing them to release heat. This can allow for rapid rewarming of cryopreserved tissue (necessary to prevent damaging ice formation) at unprecedented uniformity, preventing the thermo-mechanical stresses otherwise encountered.

Precise activation of cryoprotection

Organ banking breakthroughs could be achieved by technologies that enable rapid changes in physiological conditions as organs are cooled or warmed. For instance, rapid addition or removal of cryoprotective agents could help confine their chemical activity to temperatures at which they are beneficial.[31] Likewise, rapid, controlled changes in solution viscosity are needed to allow cryoprotective agents to be added at high temperatures while preventing ice formation at low temperatures.[32] Nanoparticles have been used to overcome analogous challenges in other contexts, such as delivery of toxic chemotherapeutics in cancer treatment.[33] Similar strategies could be used to trigger the release of agents that catalyze polymerization or de-polymerization (thereby controlling solution viscosity) or synthesis of cryoprotectant small molecules from non-toxic precursors.[32]

Real-time imaging of ice crystallization

As discussed above, the flexibility of many nanoparticles and their properties as contrast agents make them ideal for 3-dimensional imaging of tissues in real time.[3] A large diversity of ice-binding molecules have been discovered,[34] creating many opportunities to use decorated nanoparticles to detect ice crystallization. Excessive ice crystallization can be fatal to cryopreserved tissues and the foremost organ banking methods seek to avoid it altogether.[31,35,36] Nanoimaging of ice formation could be used to direct cooling and heating when unsafe ice concentrations are detected, evaluate the success of banking procedures prior to transplantation, and advance our fundamental understanding of tissue cryopreservation.

Real-time imaging of cryoprotection

Cryopreservation requires adding cryoprotective agents (CPAs) that typically are toxic at higher temperatures but protective at lower temperatures.[37] A foundational strategy to overcome this problem is to minimize the time that biological materials are exposed to CPAs before cooling.[38] This is a challenge in organ banking because of uncertainty regarding when CPAs have completely permeated all tissues; diffusion is often non-uniform.[31] Nanoimaging has been suggested as a means to track CPA permeation in real time, allowing both the exposure time and concentration to be kept to minimal levels required for cryopreservation.[32]

Stress tolerance enhancement

Nanotechnology has provided novel strategies to therapeutically intervene in cell death signaling, enhancing tolerance to stresses that can otherwise result in tissue damage. For instance, researchers have found that conjugating nanoparticles to modified Annexin V, a signaling factor that normally promotes cell death by apoptosis, has protective effects.[39] Inhibition of apoptosis has been shown to be protective during cryopreservation,[40] ischemia,[41] and mechanical stress,[39] all of which are challenges which must be overcome in organ banking.[11,27]

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REFERENCES

1. President Obama. *Fact Sheet: Obama Administration Announces Key Actions to Reduce the Organ Waiting List.* Washington, D.C.; 2016.
2. Manuchehrabadi N, et al. Improved tissue cryopreservation using inductive heating of magnetic nanoparticles. *Sci Transl Med.* 2017;9(379).
3. Tasciotti E, et al. The Emerging Role of Nanotechnology in Cell and Organ Transplantation. *Transplantation*. 2016;100(8):1629-1638.
4. Sebastian Giwa, et al. The promise of organ and tissue preservation to transform medicine. *Nat Biotechnol*. 2017;35(6):530-542.
5. Hedlund A, et al. Gd_2O_3 nanoparticles in hematopoietic cells for MRI contrast enhancement. *Int J Nanomedicine*. 2011;6:3233-3240.
6. Toy R, Bauer L, Hoimes C, Ghaghada KB, Karathanasis E. Targeted nanotechnology for cancer imaging. *Adv Drug Deliv Rev.* 2014;76:79-97.
7. Giljohann DA, Mirkin CA. Drivers of biodiagnostic development. *Nature.* 2009;462(7272):461-464.
8. Tokel O, Inci F, Demirci U. Advances in Plasmonic Technologies for Point of Care Applications. *Chem Rev.* 2014;114(11):5728-5752.
9. Yan L, Zhang J, Lee C-S, Chen X. Micro- and Nanotechnologies for Intracellular Delivery. *Small.* 2014;10(22):4487-4504.
10. Wang ZL, Wu W. Nanotechnology-Enabled Energy Harvesting for Self-Powered Micro-/Nanosystems. *Angew Chemie Int Ed.* 2012;51(47):11700-11721.
11. Lewis JK, et al. The Grand Challenges of Organ Banking: Proceedings from the first global summit on complex tissue cryopreservation. *Cryobiology.* 2016;72(2):169-182.
12. Khush KK, Menza R, Nguyen J, Zaroff JG, Goldstein BA. Donor predictors of allograft use and recipient outcomes after heart transplantation. *Circ Heart Fail.* 2013;6(2):300-309.
13. Sack K. In discarding of kidneys system reveals its flaws. *N.Y. Times* (2012).
14. Khush KK, Zaroff JG, Nguyen J, Menza R, Goldstein BA. National decline in donor heart utilization with regional variability: 1995-2010. *Am J Transpl.* 2015;15(3):642-649.
15. Israni AK, Zaun D, Rosendale JD, Snyder JJ, Kasiske BL. OPTN/SRTR 2012 Annual Data Report: deceased organ donation. *Am J Transplant.* 2014;14 Suppl 1:167-183.
16. Luisa Quinti, Ralph Weissleder and, Tung C-H. A Fluorescent Nanosensor for Apoptotic Cells. *Nano Lett.* 2006;6(3):488-490.
17. Sosnovik DE, et al. Molecular MRI Detects Low Levels of Cardiomyocyte Apoptosis in a Transgenic Model of Chronic Heart Failure. *Circ Cardiovasc Imaging.* 2009;2(6):468-475.
18. Miao R, et al. Silicon Nanowire-Based Fluorescent Nanosensor for Complexed Cu 2+ and its Bioapplications. *Nano Lett.* 2014;14(6):3124-3129.
19. Pianta TJ, Peake PW, Pickering JW, Kelleher M, Buckley NA, Endre ZH. Evaluation of biomarkers of cell cycle arrest and inflammation in prediction of dialysis or recovery after kidney transplantation. *Transpl Int.* 2015;28(12):1392-1404.
20. Farr TD, et al. Imaging Early Endothelial Inflammation Following Stroke by Core Shell Silica Superparamagnetic Glyconanoparticles That Target Selectin. *Nano Lett.* 2014;14(4):2130-2134.
21. Bruinsma BG, Sridharan G V, Weeder PD, et al. Metabolic profiling during ex vivo machine perfusion of the human liver. *Sci Rep.* 2016;6:22415. doi:10.1038/srep22415.
22. Park J-H, Byun J-Y, Shim W-B, Kim SU, Kim M-G. High-sensitivity detection of ATP using a localized surface plasmon resonance (LSPR) sensor and split aptamers. *Biosens Bioelectron.* 2015;73:26-31.
23. Fishman JA, Grossi PA. Donor-derived infection--the challenge for transplant safety. *Nat Rev Nephrol.* 2014;10(11):663-672.
24. Srinivasan A, et al. Transmission of rabies virus from an organ donor to four transplant recipients. *N Engl J Med.* 2005;352(11):1103-11.
25. Inci F, et al. Multitarget, quantitative nanoplasmonic electrical field-enhanced resonating device (NE2RD) for diagnostics. *Proc Natl Acad Sci U S A.* 2015;112(32):E4354-63.
26. Tokel O, et al. Portable microfluidic integrated plasmonic platform for pathogen detection. *Sci Rep.* 2015;5:9152.
27. Organ Preservation Alliance. Organ Bioengineering and Banking Beta Roadmap. *Organ Preservation Alliance*; 2015.

REFERENCES

28. Multi-Agency Tissue Engineering Sciences (MATES) Interagency Working Group. *MATES: Advancing Tissue Science and Strategic Report.* (Multi-Agency Tissue Engineering Sciences (MATES) Interagency Working Group, ed.); 2007.
29. Michael L. Etheridge Leoni Rott, Jeunghwan Choi, Birgit Glasmacher & John C. Bischof YX. RF heating of magnetic nanoparticles improves the thawing of cryopreserved biomaterials. *Technology.* 2014;2(3):229-242.
30. Eisenberg Bischof, J.C., Rabin, Y. DP. Thermomechanical Stress in Cryopreservation Via Vitrification With Nanoparticle Heating as a Stress-Moderating Effect. *J Biomech Eng.* 2016;(138).
31. Fahy GM, et al. Cryopreservation of organs by vitrification: perspectives and recent advances. *Cryobiology.* 2004;48(2):157-178.
32. Fahy G. Personal communication.
33. Tietze R, et al. Magnetic nanoparticle-based drug delivery for cancer therapy. *Biochem Biophys Res Commun.* 2015;468(3):463-470.
34. Davies PL. Ice-binding proteins: a remarkable diversity of structures for stopping and starting ice growth. *Trends Biochem Sci.* 2014;39(11):548-555.
35. Fahy, G.M., et. al. Vitrification as an approach to cryopreservation. *Cryobiology.* 1984;21(4):407-426.
36. Taylor Song, Y.C., Kheirabadi, B.S., Lightfoot, F.G., Brockbank, K.G.M. MJ. Vitrification fulfills its promise as an approach to reducing freeze-induced injury in multicellular tissue. *Adv Heat Mass Transf Biotechnol.* 1999;HTD-Vol. 3:93-102.
37. Fahy GM. Cryoprotectant toxicity neutralization. *Cryobiology.* 2010;60(3 Suppl):S45-53.
38. Pegg DE, Wang L, Vaughan D. Cryopreservation of articular cartilage. Part 3: the liquidus-tracking method. *Cryobiology.* 2006;52(3):360-368.
39. Chen HH, et al. Cytoprotective nanoparticles by conjugation of a polyhis tagged annexin V to a nanoparticle drug. *Nanoscale.* 2015;7(6):2255-2259.
40. Baust JM, Vogel MJ, Van Buskirk R, Baust JG. A molecular basis of cryopreservation failure and its modulation to improve cell survival. *Cell Transpl.* 2001;10(7):561-571.
41. Jiang X, Guo C, Zeng X, Li H, Chen B, Du F. A soluble receptor for advanced glycation end-products inhibits myocardial apoptosis induced by ischemia/reperfusion via the JAK2/STAT3 pathway. *Apoptosis.* 2015;20(8):1033-1047.

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