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Designing Nanostructures at the Interface between Biomedical and Physical Systems

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Build a System that will Detect Disease in vivo and Report Back Results

Focus Group Summary

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Summary:

Remember the 1966 Science Fiction movie *Fantastic Voyage* where the protagonists travel inside the body to remove a blood clot?

Thirty-nine years later, Focus Group 3 did not quite suggest sending people inside the body to detect disease. Instead, the group suggested sending nanoparticles. The particles would bind to certain disease specific target molecules in the blood stream, on cell surfaces or even inside cells. They would "recognize" which molecules they encountered because their surfaces would be specifically and irreversibly changed by binding to a specific target molecule. The particles could then be collected and analyzed after excretion in the urine so doctors could check what's wrong inside the body. While the initial

concept was straightforward, several details needed to be clarified.

One question is how to introduce the particles into the blood stream. Various methods of introducing the nanoparticles into the body were discussed: taking a pill, inhalation, or entry through the skin via a patch or injection. In addition, the particles would have to be less than 5 nm in diameter so they can be excreted through the kidney. The material of which they are made has to be both biocompatible and inert. The group considered gold and diamond, both of which are already approved by the FDA for use inside the human body, good candidates.

The next challenge was to make sure the particles would not be rejected by the immune system. Here the water-soluble polymer Poly (Ethylene Glycol), or PEG, could be used because it has been shown not to be recognized by the immune system. The particles would then have to specifically detect certain molecules that indicate disease states. Initially, the group considered particles that detect specific disease targets and then send a signal to a monitoring device outside the body.

This solution, however, could be very costly; so the group decided to solve the problem without a monitoring device. For example, an additional PEG or related cross-linked hydrogel layer could be placed on the outside of the particle. The particle would shed this layer once it binds a specific target molecule. One possible mechanism is to place ligand-receptor pairs in the outer layer. Binding of a target molecule would replace the ligand in the outer layer and cause it to fall off.

Alternatively, certain molecules in the outer layer could be cleaved by a target molecule. One example is metalloproteases found in atherosclerotic plaques that can cleave proteins. Such a cleavage would cause the nanoparticle to shed its outer hydrogel layer after it encountered metalloproteases.

Whatever the shedding mechanism, the loss of the outer layer would give the nanoparticles a memory as to which molecules they encountered in the body. The presence of particles collected in the urine without the outer layer would then indicate to a physician that the particles bound to their specific target.

One of the great advantages of such a nano-approach is that one could place a "nanotrailmix" of particles specific for hundreds--if not thousands--of different molecular targets in the body at the same time. The term "trailmix" applies because different particles would have different outside layers depending on their molecular targets, similar to "peanuts that have different kinds of salt on them," as one group member put it. It appears that the group came up with the term nanotrailmix for the first time, illustrating the innovative nature of the focus group discussions.

However, the trailmix-approach poses a problem: How do you know which particle missing its outer layer encountered which target molecule? To find out, particles with different specificities could be "bar-coded" to make them identifiable. One way to do this is to place oligonucleotides--or short DNA molecules--in the inner PEG layer left behind. The oligos could then be used to hybridize to their counterparts on a DNA microarray chip. Alternatively, one could use a RAMAN active substance for the inner PEG layer. Different substances could emit different wavelengths after excitation with laser light, and this could serve as a particle ID.

So which targets could such a system detect? The group decided to approach the problem in three stages of developing the technology, with the most accessible targets addressed first, in Stage 1. Stage 1 targets are molecules accessible from the bloodstream where the nanotrailmix is circulating anyway, such as Prostate Specific Antigen, a protein expressed in the prostate thought to indicate the presence of cancerous cells. Other possible targets are metalloproteases in atherosclerotic plaques, although these may be too unspecific, because they are elevated in the vessels of inflammatory lesions and cancers, and probably other diseases as well, one group member said. However, that group member added, inflammation, cancer and pre-malignant lesions are recognizable from more specific vascular changes. For example, tumor blood vessels express molecular markers that can even be specific for a given type of cancer.

Stage 2 would be to detect targets outside the peripheral blood system, in the intercellular space. As long

as the nanoparticles are smaller than 50 nm, they could leave the cardiovascular system through pores. Possible targets are cancer cells expressing altered receptors or, in the brain, amyloid plaques in Alzheimer's patients.

The most difficult region for the particles to reach is the inside of cells--Stage 3--to detect, for example, such cancer-specific molecule variants as mutated p53. To enter cells, a nanoparticle could contain a molecule in the outer layer that can induce uptake into the cell. Such molecules are already known; for example, a peptide from the TAT protein of the HIV virus can enter into cells and is capable of taking a payload, even a nanoparticle, with it.

One major target that can be monitored by a nanotrailmix approach in all three stages of development is infectious agents such as viruses, bacteria or parasites, some of which can be present in the bloodstream as well as on cell surfaces or inside cells. A nanotrailmix has the "nanoadvantage" of early and rapid identification of such infectious agents, whereas the conventional approach involves growing cultures before the infectious agents can be identified. This advantage comes to play at all three stages. For example, while current blood tests can already measure molecules in the blood without any nanoparticles, a nanotrailmix would enable doctors to measure a large number of possible targets at the same time. "A nanotrailmix approach gives you the ability of massive parallelism," said one member of the group.

It is unclear, however, where in the body the particles encounter their target molecules once they are in the urine. Imaging could be used to specifically look for the location of these target molecules, perhaps even using nanoparticles coupled to contrast agents and then localized inside the body.

Many more hurdles and gaps in knowledge remain to be overcome. One is that there is little known about the biocompatibility of nanoparticles. The strategy for the particles to exit cells is unclear as well. It may also be difficult to amplify the signal coming from the oligonucleotide barcodes. One way to label them is fluorescent molecules, but with only a few fluorescent molecules per nanoparticle, the signal may be too weak. Another concern is the potential environmental impact of the particles.

The group also discussed the potential societal impact of the nanotrailmix approach. One group member pointed out that continuous monitoring with nanoparticles could make people overly concerned about their health. "Maybe you are going to have some serious hypochondriacs," that group member noted. The problem could be avoided if hospitals analyze the particles and alert patients only if they find something. Another problem is that every single particle type might require separate FDA approval. "Maybe your grandchildren will see this technology," a group member said.

Group members agreed that the societal impact of the nanotrailmix technology would be positive in that it would be easily deployable to developing countries, and it would enable the collection of new research data for epidemiological studies. However, there was concern about privacy issues, with the collection of so much data, and about possible false negative or positive results. The exact cost of care using the nanotrailmix technology is unknown, but some in the group said it might cost less to determine the kind of cancer a patient has than such current invasive approaches as surgery.

Some group members indicated that there is a long way to go before the technology discussed becomes realized. "This is really hard, and that's fine; it's not going to happen over night," commented one group member. "We need to be very careful what we promise."

The challenge is to incorporate sufficient specificity and functionality into the particles, but still conform to the size requirements, one group member said, adding that the functional layers will likely make the particle larger than 5 nm. "That's not to say it can never be done, but these are the kinds of challenges that you are going to have to figure out," the group member noted, adding that one could use self-destructive polymers so particles bigger than 5 nm would eventually go away; or one could use composite particles that fall apart at some point. It was also suggested to keep the particles circulating so they could be bigger than 5 nm. After encountering their targets, the particles could change color or become fluorescent while in circulation, so as to be detectable by optical methods, for example, through the skin

or eye.

However big the challenges are, many in the group said that simply discussing such issues in the focus group gave them novel insights from other disciplines. "It was intriguing and enthralling to brainstorm with people of such diverse backgrounds," one group member said. "The networking through brainstorming was a very effective mechanism."