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Encapsulated Bacteria with a Light-Repressed Deadman Switch for Liver Gene Delivery

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Posted Date: 10 May 2024

doi: 10.20944/preprints202404.1631.v3

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Encapsulated Bacteria with a Light-Repressed Deadman Switch for Liver Gene Delivery

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Abstract: Adeno-associated viruses (AAVs) have been used for liver gene therapy. Hemgenix and Roctavian are AAV-based treatments for hemophilia B and A, respectively. They cost $3.5 million and $2.9 million per dose, respectively. While AAV vectors may eventually be cheaper to mass produce, a bacterium-based DNA delivery system might be much cheaper for patients. Also, this approach would allow for the delivery of much larger DNA packages. Such a bacterial system may now be feasible, and a prototype for the liver could possibly be developed immediately.

Keywords: bacterial capsule; caged luciferin/luciferase; CY3PA; light-activated allosteric protein switch; Deadman switch; bactofection

Introduction

Adeno-associated viruses (AAVs) can only encode ~5 kb of DNA maximum, which is not sufficient to cure certain individuals with genetic disorders wherein long stretches of nucleotides are affected, e.g., at least some cases of Duchenne muscular dystrophy [1–6].

According to Dr. Anzalone *et al.*, "…in principle prime editing can correct up to ~89% of the 75,122 pathogenic human genetic variants in ClinVar…"[7]. New prime editing-based techniques can almost surely cover even more variants [8]. However, multiple proteins/RNAs/DNA molecules (or at least a single, large DNA molecule) may be required in certain cases when more complex molecular machinery must be employed, e.g., if heterochromatin is an issue.

Also, the cost of producing sufficiently high titers of AAV vectors and other viral vectors for therapeutic purposes is very steep [9–11].

Lipid nanoparticle (LNP)-encapsulated CRISPR ribonucleoproteins (RNPs) have been successfully utilized for gene delivery and editing in the liver and somewhat in the lungs [12,13]. However, current LNP formulations at least cannot effectively reach or target cells in the central nervous system (CNS), heart, or kidneys after intravenous injection[13,14]. Additionally, the larger the LNP - the less effective it is at extravasation. Thus, it cannot encapsulate large cargos unless targeted to the liver or spleen. Finally, LNP production costs are still substantial, and scaling up the production of new ionizable lipids is not that facile [15–18].

Intrathecal or intracerebroventricular administration of RNPs, as well as direct intraparenchymal injection of RNPs into the striatum, does not result in widespread CNS editing [19].

A synthetic bacterial vector with low immunogenicity that only delivers to particular organs or organ systems would be of use for multiple reasons:

1. It would be cheap.
2. It could deliver large DNA constructs to use as homologous repair templates or large serine recombinase/CRISPR transposase cargo for individuals with genetic disorders wherein long stretches of nucleotides are affected.
3. May be able to extravasate at least moderately efficiently in most anatomical locales - and then specifically proliferate in the target organ or organ system [20,21]. (If necessary - intraparenchymal injections could be employed.)
4. Flagellar motility of the vector allows for autonomous, widespread delivery throughout the organ or organ system [22].

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Prototype for the Liver

Recently, a new system was developed for the programmable encapsulation of microbes to attenuate the immune response and enhance multifocal tumor therapy. An *Escherichia Coli* Nissle 1917 or a *Salmonella* Typhimurium vector could be encapsulated to prevent cytotoxic protein secretion from affecting the tissue environment and substantially attenuate the immune response [23]. It can also be myristoylation negative [24]. One could employ an anti-sepsis small molecule, at least in a mouse model [25]. Dexamethasone would be used to further suppress the immune response and induce the expression of CY3PA in the liver. The bacterial vector could then be administered. Finally, one would periodically administer a caged luciferin molecule that is uncaged by CY3PA [26]. The bacterial vector would express firefly luciferase and have a Deadman switch that is sensitive to light [27–31]. Bacteria that stray outside of the liver, where the luciferin is uncaged, would quickly stop replicating and lyse.

When the bacteria in the liver reach a high enough population level (either visualized with an MRI-based reporter gene [32] or just after an experimentally-determined period of time), they can be induced via small molecule to produce a large quantity of CRISPR RNPs[19,33] and lyse.

Theoretically, *E. coli* Nissle 1917 is endotoxin-free. However, to prevent possible issues from mass lysis, perhaps the small molecule should stop motility immediately - while the gene circuit for lysis can be noisy [34].

Another option would be to use a second Deadman switch; a molecule that the bacteria are exposed to in solution prior to intravenous injection would be gone after injection, and eventually they will just stop replicating and lyse naturally.

Bactofection is a method that has been used for DNA transfer to target cells. It involves invasion of a facultative intracellular bacterium into the a target cell, endosomal rupture, and finally lysis [35]. One of the primary roadblocks to bactofection in vivo has been the immune response to the bacteria.

Notably, only two genes may be required to enable this strategy using *E. coli* Nissle 1917: the *Yersinia pseudotuberculosis* invasin and listeriolysin O [36,37].

Having a thick capsule may hinder hepatocyte uptake, however [38]. If so, the bacteria could perhaps divide several times after halting capsule production and overexpressing an eliminase to shed its capsule [39], enter hepatocytes [40], escape the endosome or vacuole[36,37,41], and then lyse.

To increase the efficiency of bactofection, the *Listeria monocytogenes actA* promoter can be used to drive phage lysin production when the bacteria enter the cytosol of target cells [42]. Using a linear plasmid in combination with NLS-containing proteins that bind said plasmid could substantially increase the efficiency, as linear DNA may be taken up more easily through nuclear pore complexes [43,44]. Moreover, it may be possible to employ a mechanism involving asymmetric division of the vector inside target cells, wherein one of the progeny cells remains in a vacuole and the other lyses to release its cargo [45,46]. The other progeny cell could continue to asymmetrically divide until at least one copy of the DNA construct reaches the host cell nucleus, at which point expression of an artificial gene product would cause it to lyse.

Finally, to strengthen this system, prior to asymmetric division, the bacterium could potentially be allowed to undergo replication up until a tolerable copy number, then being restrained by quorum sensing.

Bacterial entry and release of a replicating RNA vector may also help in the case of RNP delivery, to enhance gene editing efficiency [47–49].

For diseases like Hemophilia A and B, not every hepatocyte nucleus would need a copy of the gene construct in order to achieve a curative status, which is helpful with regard to bactofection efficiency and immunogenicity.

Other Organs and Organ Systems

We could identify other enzymes specific to an organ or organ system and develop caged luciferins for those as well. Alternatively, the enzyme could be an organ or organ system-specific extracellular protease, for example. The protease could directly or indirectly activate regulated intramembrane proteolysis [50]. Or, it could cleave a pro-peptide that activates a two-component

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regulatory system [51]. The benefit of either of those approaches is that an exogenous small molecule would not be required for the continued survival and replication of the vector.

Bacteria can also cross the blood-brain barrier after intravenous injection[21,52].

There is a caged luciferin that can be uncaged in the brain - but it also is uncaged in the kidneys [53]. A better strate[gy, for now at least, may be to administer a small molecule that is blood-brain barrier-impermeable and that kills any of the synthetic bacteria in the periphery.

In a paper by Dr. Antas *et al*., it was said that "Although nonviral NPs may tackle the main limitations of their viral counterparts, they have thus far failed to compete with the transduction efficiency achieved by viral vectors in the retina"[54]. Bactofection might be better than AAVs for ocular genetic diseases [55,56]. A Deadman switch could be used to allow limited bacterial replication in the eye via a small molecule present in an eye-drop solution or micro-drug reservoir [57].

Identifying tissue-specific enzymes should not be very difficult nowadays [58].

Conclusions

Encapsulated bacteria with a light-repressed Deadman switch could be used for liver gene delivery. They may be much cheaper than AAV-based liver gene therapies, and even LNPs. It would also allow for the delivery of large DNA packages. In the future, other organs that are harder to reach with gene therapy or editing components, like the heart or brain, could be treated with this therapy.

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 doi:10.20944/preprints202404.1631.v3

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