Slow-release nano-pills for mosquitoes for interrupting malaria transmission

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I. Fundamental idea:

To interrupt malaria transmission at the critical time point in the parasite life cycle, the mosquito stage, by luring mosquitoes into ingestion of novel anti-malarial slow-release nano-pills.

Rolling back and eradicating malaria is a major goal of global health, but current efforts focusing on bed nets, mosquito control, rapid diagnosis and treatment, as well as vaccination, are only partly successful and are challenged by cost, by resistance of mosquitoes to insecticides and of malaria plasmodia to anti-malarial drugs. Resistance development is associated with exposure of a large number of mosquitoes to insecticides or of plasmodia to anti-malarial drugs: In the life cycle of plasmodia, an infected human harbours about 10^9-10^{12} plasmodia, leading to exposure of a large number o plasmodia to anti-malarial drugs in current treatment strategies, thereby facilitating resistance development. In contrast, the lowest number of plasmodia during its life cycle is during the insect vector stage (1-5 plasmodia/mosquito), and the chance of resistance development might be drastically reduced if plasmodia elimination succeeds at this very time point.

The unconventional approach of treating malaria in the vector stage in the mosquito by anti-malarial slow-release nano-pills *for mosquitoes* (as opposed to treating malaria in the human body) could therefore interrupt malaria transmission in a way that

- does not induce insecticide resistance in mosquitoes, and
- minimizes the chance of anti-malarial drug resistance development in plasmodia.
- abolishes drug side effects of anti-malarial drugs in humans, what will allow the use also of alternative or more toxic anti-malarial drugs.

A single mosquito feed on nano-pill spiked food might eliminate plasmodia present in the insect at this moment only, but by designing slow release/long persistence nano-pills, transmission might be blocked in the best case during the entire life time of a mosquito after a single meal. We have proven the concept of slow-release nanopills in other contexts (Benhaim,Broz,Hunziker 2007-2012).

Feeding habits of mosquitoes transmitting malaria are well studied: Female Anopheles gambiae in their first 5 days of life prefer sugar feeding to human blood meals[1] and then switch their preference. Mosquitoes find humans for blood meals by sensing CO2, temperature, smells (“Limburger cheese” flavour/hexanoic acid) and light[2], offering modalities that can be combined into attractive non-living feeding objects for mosquitoes, either in the sugar feeding stage or the blood meal stage.
Anti-malarial drugs with potent effects on mosquito stages of plasmodia are known: cycloheximide and atovaquone are particularly promising payload candidates[3].
Nanomaterials are suited as drug carriers[4], and allow to tailor release kinetics of a payload towards “slow-release” over a prolonged time in a very confined space, such as within a single cell[5] or within an insect gut (this proposal), and can be surface functionalized for prolonged cell adhesion. High yield/low cost production of such nanomaterials is feasible in our experience, a prerequisite to render such an approach cost-effective in the global fight against malaria. If necessary, adding additional functionality to nanomaterials like tissue adherence or digestive enzyme triggering is feasible.

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[1] Foster WA. Bull Entomol Res. 2004; 94:145-57
[2] Knols BGJ. Parasitology Today 1996 ; 12 : 159–161
[3] Delves M PLoS Med 2012; 9: e1001169.
[4] Broz P. Nanotechnologies for targeted delivery of drugs. In: Kumar, *Nanomaterials for Medical Therapy*. Wiley-VCH; 2007
[5] Ben-Haim N. Nano Letters 2008;8: 1368–1373

Hypotheses to be tested:
1) Mosquitoes can be tricked into taking up nano-pills that slowly release a drug or drug-like model molecule (to be tested in phase I of the project).
2) Plasmodia transmission can be stopped in the mosquito stage through nano-pills that slowly release anti-malarial drugs (phase II of project)

II. How to test/work packages
A) Nano-pills will be synthesized based on our established synthesis protocols. Slow-release polymer nanocarriers will be covalently tagged with a fluorescent dye to enable tracking of the nano-pill. A midgut enzyme-resistant copolymer will be chosen initially, namely a polydimethyl-siloxane-polymethyloxazoline synthesized by ring-opening polymerization, with a biodegradable copolymer (polylactic acid-polyethylene glycol) as alternative (made available by our chemist according to in-house protocols, including thorough characterization of nanomaterials by NMR, UV-VIS, DLS, EM, zeta potential).
B) As model payloads for slow drug release, fluorescent dyes of a different wavelength, having similar physicochemical properties (solubility, size) as anti-malarial drugs that are known to be potent inhibitors of the mosquito stage of plasmodia, will be loaded into the nano-pills using established loading protocols. Atovaquone, being intrinsically fluorescent and a potent antimalarial in the mosquito stage (Delves 2012) will be studied with particular emphasis. Cycloheximide, another potent mosquito stage anti-malarial drug, will be matched by fluorophores with similar properties for fluorescence analysis.
C) Mosquito feeding experiments will be performed using standard sugar media spiked with anti-malarial nano-pills, including suited negative controls, as well as controls with soluble fluorophores / anti-malarials in the absence of nano-pills. Source of anopheles mosquitoes is a malaria insectarium within an established collaboration.
D) Using standard mosquito micro-dissection techniques and fluorescence microscopy, the fluorescent nano-pills, which have proven to be visible in biologic tissue in our prior work, will be localized in crop, midgut and hindgut by fluorescence microscopy and subjected to semi-quantitative optical analysis using our established protocols. Nano-pill location and content release over time will be studied at various time points after feeding.
E) Nanopill-enriched semi-solid macroscopic feeding objects for mosquito feeding will be assembled and will be equipped with simple chemical and/or physical attractors for anopheles (Foster 2004), suited for distribution in the environment, keeping the design simple enough for future mass production.
F) Additional options for a later stage include enzyme activated drug release through a midgut-degradable polymer and ligand binding of the nano pill to midgut cells through carbohydrate binding to prolong persistence of the nano-pill in the gut.

Budget: 1 Doctorand incl Consumables: flat rate 65kFr * 4 years = 260kFr.

Essential data to be acquired in phase I:
a) feasibility of nano-pill feeding to mosquitoes
b) location of nano-pills in mosquito midgut after feeding
c) time course of nano-pill concentration in mosquito midgut
d) time course of payload release from nano-pills,
all assessed by fluorescence imaging

Steps in phase II
a) optimize nano-pill chemistry towards optimal efficacy and minimal price
b) quantitative analysis of nano-pill deposition and anti-malarial payload, and of drug release over time by suited method (e.g., gas chromatography, mass spectroscopy)
c) study the efficacy of transmission blocking by nano-pill in laboratory context (established collaboration with malaria insectarium)
d) long term: field testing of fluorescent nano-pill uptake by suited feeding objects in an area with a high mosquito density (established field collaborations)