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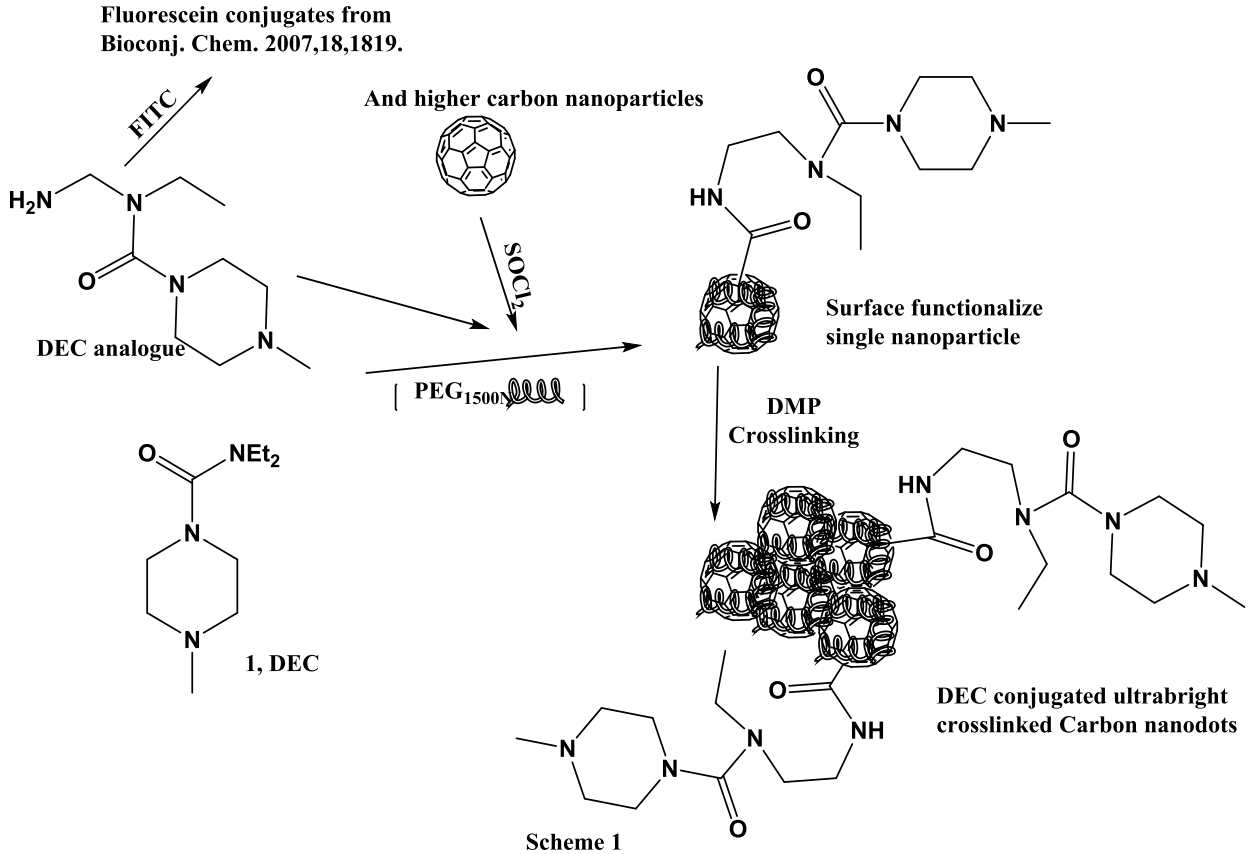
Onchoflash: A rapid noninvasive strategy for the detection of Onchocerca microfilaria in the skin

Section I. The African Programme for Onchocerciasis Control (APOC) and national onchocerciasis control programs in Africa are currently attempting to move from the control of onchocerciasis as a clinical disease, to a push to eliminate onchocerciasis in Africa. This may be feasible, but is a challenging goal. One of thechallenges to achieve this goal is to be able to monitor the prevalence and intensity of infection as worm burdens decrease with repeated rounds of Mectizan® treatment. Nodule palpation can be used as an approximation for prevalence for mapping purposes, before mass drug administration is commenced. However, nodule palpation is poorly predictive once people have been on Mectizan for a few rounds and can be quite misleading as microfilarial loads decrease during control programs. Monitoring for the effectiveness of Mectizan treatment has been done in the past, at the individual subject level, by skin snip microfilarial counts. Taking skin snips is invasive and somewhat painful. In endemic areas, people are increasingly resistant to having skin snips taken. Furthermore, as manifestations of the disease, such as pruritis and eye disease, decrease with movement towards elimination, individuals in endemic areas can be expected to become even more reluctant to allow skin snips to be taken so that progress towards elimination can be monitored. The DEC, 1, patch test has been used as an alternative to skin snipping to monitor for the presence of microfilariae in the skin. However, the method is poorly quantitative and because it requires that the patient return the next day to have the patch removed and the skin under the patch examined there are problems with compliance, with some patients removing the patch before returning for examination and other patients not returning for the reading of the 'patch'. Because the current DEC patch test relies on an inflammatory response under the patch, which causes local irritation, there is a tendency for some patients to remove the patch irritant before it is properly read by an experienced monitor. What is urgently needed is a rapid, non-invasive, semi-quantitative method for assessing skin microfilarial loads. In order to achieve elimination of onchocerciasis, there is an increased effort, supported by the BMGF, to develop new anti-filarial pharmaceuticals, including possible macrofilaricides. Currently, novel anti-filarial drugs to be tested against Onchocerca volvulus require that the drug effects be assessed by skin snip microfilariae counts and nodulectomy to assess the viability of adult and microfilarial stages of the parasite. Both nodulectomy and skin snip sampling are highly invasive, painful and have a risk of secondary infections resulting from the surgical interventions. Repeated microfilarial level assessment provides a readout of both microfilaricidal effects of an experimental filaricide and anti-adult filarial effects, either resulting from the adults being killed (macrofilaricidal effects) and/or anti-fecundity effects (prolonged inhibition of the adult worm's ability to produce microfilariae). Here we propose just such a rapid, non-invasive, semiquantitative method for assessing microfilarial loads which will be an invaluable tool to assist with the development and use of novel anti-filarial pharmaceuticals which could be employed in the battle to eliminate onchocerciasis.

The Innovation : In the course of developing new fluorescent probes for DEC targets in filiarial worms (Bioconj. Chem. 2007,18,1818) it was found that the labeled drug accumulates in many sites of all worms. These conjugates contain fluorescein and rhodamine which although useful for confocal microscopy are of limited application for diagnostics due to sensitivity, photobleaching, and relatively low quantum yield. Our idea is to adapt new types of ultra-bright fluorescent probes in these DEC conjugates, ones which will allow for a much brighter, stabile, non-bleachable fluorescent probe for the rapid noninvasive detection of microfilaria in skin. These probes are anticipated to be bright enough to allow for the use with simple hand held detectors pressed up to the skin to give a readout of microfilarial load. Our working term for this strategy is Onchoflash.

Section II. The key to Onchoflash is that new non-toxic carbon nano-dots are now available (Small, 2013,9,545.). These outcompete the cadmium selenide fluorescent probes and avoid all of the issues with the toxicity of cadmium and selenium. The carbon nano-dots fluoresce in the green, em = 440 nm, and are readily excited with blue radiation ~400 nm. They have proven bright enough for use as subcutaneous probes in mice. The synthesis of these probes is shown in Scheme 1. Briefly, the carbon nanoparticles will be activated with OSCl2 treatment and followed by binding of a mixture of N-acetylated PEG, PEG1500N, and the amino DEC analog. Note that a Fullerene is shown for simplicity in Figure 1 but larger particles will be used. The ratio of DEC/PEG surface covering will need to be optimized for worm affinity, correct fluorescence, and aggregation properties. If need be longer linker groups can be added to the amino DEC analog to improve parasite labeling. The dimethylpimelimidate (DMP) crosslinking step will create nanoparticles of larger sizes and with correct DEC analog loading there should be DEC at numerous surface sites. After cross linking an additional round of optimization for fluoresence, and worm affinity will be performed as a prelude to testing the new Onchoflash probes in animal models. Initially, the way the Onchoflash test is to be conducted is with the following steps. First a surface patch will be applied to the upper arm using a bandaid affixed gauze as in the current test. The gauze in this test will be loaded not with DEC but with the onchoflash probe and a mobilizing agent such as DMSO to accelerate the probe uptake. To read the result of fluorescent probe labeling the bandaid and gauze will be removed, after 30 minutes, and the surface briefly cleansed. A hand held spectrofluorometer will be pressed at that spot to detect and count the emitted radiation From the probe. These carbon nanoparticles are so bright that it may be possible in cases of high parasitemia to see the green signal with the naked eye. More significantly though are the cases of low parasitemia where such a sensitive detector can be used semiquanititatively. Microfilaria are DEC accumulators and so the fluorescent probes should concentrate and be immobilized in the worm. Un bound probe is expected to diminish in concentration by a combination of diffusion and clearance by immune system.



Scheme 1