Published in final edited form as:

J Biophotonics. 2016 July ; 9(7): 676–682. doi:10.1002/jbio.201500226.

Laser-Induced Capillary Leakage for Blood Biomarker Detection and Vaccine Delivery via the Skin

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Abstract

Circulation system is the center for coordination and communication of all organs in our body. Examination of any change in its analytes or delivery of therapeutic drugs into the system consists of important medical practice in today's medicine. Two recent studies prove that brief illumination of skin with a low powered laser, at wavelengths preferentially absorbed by hemoglobin, increases the amount of circulating biomarkers in the epidermis and upper dermis by more than 1,000-fold. When probe-coated microneedle arrays are applied into laser-treated skin, plasma blood biomarkers can be reliably, accurately, and sufficiently quantified in 15~30 min assays, with a maximal detection in one hr in a manner independent of penetration depth or a molecular mass of the biomarker. Moreover, the laser treatment permits a high efficient delivery of radiation-attenuated malarial sporozoites (RAS) into the circulation, leading to robust immunity against malaria infections, whereas similar immunization at sham-treated skin elicits poor immune responses. Thus this technology can potentially instruct designs of small, portable devices for onsite, in mobile clinics, or at home for point-of-care diagnosis and drug/vaccine delivery via the skin.

Graphical Abstract

Laser-induced capillary leakage (a) to induce extravasation of circulating molecules only (b) or facilitate entry of attenuated malaria sporozoites into the capillary (c). Skin illumination with a laser preferably absorbed by hemoglobin causes dilation of the capillary beneath the skin. The

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extravasated molecules can be sufficiently measured in the skin or guide sporozoites to enter the vessel.

1 Introduction

The logistics of directly working with blood, whether to deliver therapeutic drugs or remove analytes for examination, have always been convoluted. For example, the process of collecting, transporting, and analyzing blood samples for disease diagnosis requires coordination among the laboratory, nurses, and patients, an inherently inefficient and error-prone system. Especially in situations that require repeated blood tests to monitor disease progress and therapeutic efficacy, frequent laboratory visits for blood collections cause enough inconvenience to significantly reduce patients' compliance [1]. Meanwhile, a promising experimental malaria vaccine based on radiation-attenuated sporozoites (RAS) confers substantial protection in human volunteers [2], but demands several intravenous injections to be effective in elicitation of strong protective immunity against malarial infection [2]. Considering the challenge of immunizing large populations intravenously, regions suffering from malaria epidemics may not benefit greatly from such a vaccine. While these two problems may seem disparate, a groundbreaking solution addresses both of them, allowing indirect interactions with circulation system in a minimally invasive, facile manner.

Our recent studies have explored brief laser illumination of the skin to transiently increase capillary permeability[3,4]. Then, surface-modified microneedle arrays (MNAs) can be applied to the laser-treated skin, binding to plasma substances leaked from capillary without drawing any blood[3]. MNAs may also release drugs or vaccines into the skin for entry into permeable capillaries. The underlying principle behind the laser technology bears semblance to that of selective photothermolysis[5], except that much lower energy lasers are used to excite oxygenated hemoglobin (HbO₂) and hemoglobin (Hb) and thermally induce capillary leakage beneath the skin, without incurring any capillary rupture or blood cell extravasation. This review will discuss the novel technology's origins and its potential new applications in efficient, reliable, and rapid measurement of circulating biomarkers as well in delivering malaria sporozoite vaccines. Comprehensive reviews about microneedles or laser treatment in delivery of vaccines or drugs can be found elsewhere [6-11] and will not be the focus of the current review.

2 Selective photothermolysis and capillary leakage

Photothermolysis was used to treat hemangiomas in the early 1960s by heating precise portions of tissue to 70°C-100°C, causing protein denaturation and coagulation necrosis of excess blood vessels[12]. The treatment caused unwarranted damage to tissues surrounding target blood vessels as well. The unwanted tissue damage was greatly minimized following Anderson and Parrish's discovery of selective photothermolysis[5]. They proved it was possible to confine thermally mediated damage by radiating at an optical absorption relatively specific to the target chromophore[5]. For instance, light at 530-590 nm is preferably absorbed by Hb and HbO₂ with some overlapping of melanin. This, in line with

specific expression of abundant Hb and HbO₂ in red blood cells, raises the possibility that photoexcitation of Hb and HbO₂ could selectively ablate blood vessels[5]. Indeed, Anderson and Parrish proved the selective photothermolysis in the clinics in treating dermatological conditions characterized by excess blood vessels[13,14]. Clinicians has now used this selective photothermolysis to treat many congenital vascular lesions, including port wine stains and hemangiomas, as well as acquired lesions and other conditions for more than a decade[15]. The wide range of conditions treated, as well as a variety of lasers used in vascular treatment over the past decade, conclude safety, effectiveness, and specificity of the laser treatment.

Few advances in blood vessel targeted selective photothermolysis have arisen in the last decade, and the technology's usage has been restricted to dermatology. However the concept's potential does not end there, as recent studies have considered the potential for milder lasers to induce transient molecule extravasation, rather than rupture, of capillaries. Capillary walls consist of only a single layer of endothelial cells, so it's likely that exciting Hb and HbO₂ at lower energy levels than those used for photothermolysis could stimulate sufficient thermal dilation of capillaries to increase vessel permeability without causing any damage. Li et. al. first validated the concept by introducing fluorescein isothiocyanate (FITC) into mice intravenously, then visualizing the fluorescein leakage by intravital confocal microscopy in laser-treated skin[3]. When selecting lasers to target blood vessels, considerations include wavelength, pulse duration, fluence, and spot size [16]. The study identified that illuminating lower dorsal skin of mice with 532nm Nd:YAG laser at a pulse width 7 ns, diameter 7 mm, and an influence 0.5J/cm² caused FITC leakage immediately, peaking for 20 minutes before subsiding in ~ 1hr (Figure 1, inset). Similarly Evans blue dye, which binds to albumin after intravenous injection and becomes impermeable to blood vessels, escaped into the dermis after laser illumination. On the contrary, in the absence of laser treatment, dye was well confined within blood vessels (Figure 1). Whether tracking Evans blue dye or FITC, extravasation appeared to peak for 10-20 minutes and resolved in 1~2 hr. Histology demonstrated no significant changes in laser-treated skin other than temporary dilation of capillaries [3].

As no blood cells extravasate from the capillary, only small and macromolecules do. The dilated capillary acts as filters similar in function to the conventional blood processing to separate red blood cells from plasma, yet requiring no extra time to accomplish the task. In contrast, at least an hour is required in routine laboratory procedure of plasma preparation, during which blood clotting occurs after more than 30 min, followed by separation of blood cells from plasma by centrifugation[17-19]. Laser illumination induced “filtering” of circulating biomarkers concurs with their capture by MNAs in the skin, substantially shortening the time of the assay. The controllable leakage of blood substances into the epidermis and upper dermis holds great potential for rapid and reliable measurement of blood biomarkers in a minimally invasive, sample-free, and painless manner. The technique may also offer an alternative to intravenous injection of certain drugs.

3 Quantification of Blood Biomarkers via the skin

Onsite quantification of blood biomarkers could provide efficient and timely point-of-care diagnosis. If diseases are diagnosed and treated at earlier stages, outcomes can be improved and medical costs lowered substantially. It would also revolutionize our capacity to monitor cancers, cardiac diseases, and autoimmune diseases, as well as acute events such as various infections[20,21]. A considerable effort has been devoted to facile separation of blood cells and plasma in the past decade, such as microfluidic separations based on the Zweifach-Fung bifurcation effect[22,23]. In those cases, plasma from finger pricks is separated at a branch point in microchannels, such that blood cells travel into the channel with a higher flow rate, while plasma travels in the lower flow rate channel. However, such techniques work only with small blood samples, which limits their broad use. Concentrations of analytes are relatively low in blood and it is challenging to simultaneously detect multiple biomarkers in a single assay. Moreover, even if problems of blood collection, separation, concentration, and detection are solved individually, the difficulty of integrating all these technologies into a simple system has prevented translation of many potential point-of-care diagnostic technologies to the bedside.

Progress in sample-free biomarker detection in the skin has been made recently with surface modified MNAs [24-26]. In the skin, many plasma proteins and other solutes are constantly exchanged between capillaries and dermal interstitial fluid in a process termed “plasma turnover”[25]. Therefore, antigens or antibody-coated MNAs can capture biomarkers in the skin if they are designed to bind specifically to their targets. Several such MNAs have been developed, capable of recognizing circulating viral proteins and specific antibodies in mouse skin, allowing biomarker detection without sample processing[25]. However, current probe-coated MNAs for capturing blood biomarkers in the skin have two key drawbacks. Due to the low concentrations of most blood biomarkers in the epidermis and the upper dermis, MNAs must remain deep in the dermis for effective measurements, such that they may cause pain or break off in the skin because the deep dermis is rich in nerve endings [26]. Coffey et al. showed that the amount of plasma biomarkers was 10-fold lower than that in the deep dermis and MNAs in the epidermis or upper dermis could not sufficiently capture circulating biomarkers [25]. Only when the length of MNAs was extended into the deep dermis, circulating biomarkers could be significantly detected in 10~20 min of the assay, and the signal on the MNAs reached to a maximal level in 6 hrs of MNA application into the skin [25]. For low abundant plasma biomarkers, a few hr of MNA skin application may be required for reliable detection. Additionally, all such MNA based biomarker detection systems have demonstrated high variability, likely due to increased turnover of plasma biomarkers in uncharacterized capillaries damaged during patch application into the deep dermis [25]. As depicted in figure 2B, sufficient binding of blood biomarkers may occur only on a microneedle that is physically close to a damaged vessel like #1, but not on microneedles that are physically distant from the damaged vessel like #2 and #3 in the array. Clearly, with this technique it is challenging to measure multiple biomarkers in a single array[25], as the assay would be very vulnerable to false negatives. Recently, Lee et. al. cut a patch into three pieces followed by covalently coating a specific antibody on each piece [27]. The three pieces were then applied together into the skin of the same mice so that more

than one bio markers were detected [27]. Multiplexed microneedle arrays for truly pain-less diagnosis were indeed developed by Ng et. al. for measuring skin biomarkers but not circulating biomarkers[28]. Clearly, the large variations due to uncharacterized capillary vessel damage from microneedle penetration, along with low levels of blood biomarkers in the epidermis and upper dermis, severely limit the clinical potential of current MNA-based assays of multiple biomarkers in a single array.

Theoretically, photoexcitation of Hb and HbO₂ could allow faster and more accurate detection by increasing the permeability of all capillaries involved equally, eliminating the anomalous effects of uncharacterized capillary damage by microneedles while drastically increasing the quantity of biomarkers in the epidermis and upper dermis. This process is illustrated schematically in figure 2C and abstract, where laser illumination results in uniform and high levels of blood biomarker accumulation in the epidermis and upper dermis. Strong binding occurs on all microneedles in the array, greatly improving reliability and sensitivity of the assay (Figure 2C).

Extravasation of biomarkers from capillaries at the site of laser illumination led to a more than 1,000-fold increase in biomarker accumulation in the epidermis and upper dermis compared with controls [3]. When MNAs coated with anti-FITC antibody were applied to mice receiving FITC intravenously, detected FITC levels were substantially greater in the skin treated by 532nm laser for 5s and a fluence of 0.5J/cm², than in sham light-treated skin. As shown in Figure 2, strong and uniform FITC binding was seen in all nine microneedles in the array when applied into laser-treated skin. However, only two of the nine microneedles in the same array were FITC-positive when applied to sham-light-treated skin under similar conditions[3]. In accordance with this, brief laser illumination of the detection site increased levels of FITC detection in the upper dermis by a factor of 5 in 15~30 min assays and a maximal signal was attained in 1hr after MNA application[3], comparable to conventional immunoassays. The short period of time (< 1 hr) required for the assay offers its great potentials as point-of-care diagnosis and onsite monitoring of biological states. Similar plasma biomarker assays could be achieved only with a longer MNA in untreated skin and maximal signals on the MNAs could be obtained 6 hr after MNA application [25]. Perhaps more importantly, standard deviation in FITC levels measured by patch decreased by a factor of 6.5, so that calculation of FITC concentration from anti-FITC MNAs closely matched that measured by fluorescence spectrophotometer. In other words, laser illumination followed by anti-FITC MNA application produced highly reliable measurements of biomarkers, and could potentially allow measurement of multiple biomarkers in a single array. The uniform accumulation of high quantities of circulating biomarkers in illuminated skin robustly improved MNA-based assays.

Probe-coated MNAs not only captures small molecules like FITC, but also large, clinically relevant biomarkers like antibodies. In comparison with controls that did not receive laser treatment, MNAs covalently coated with influenza viral hemagglutinin (HA) could detect anti-HA IgG in laser-treated skin substantially more rapidly and accurately in animals immunized with influenza vaccines 4 weeks prior [3]. The anti-HA MNA can produce reliable results within 30 min in mice or 15 min in swine after laser treatment. In contrast, in the absence of laser illumination the same MNA was unable to reliably detect anti-HA IgG

in similarly immunized mice in a 2 hr assay. The standard deviation of anti-HA IgG detection in the presence of laser illumination was 1/6 of that obtained in the absence of laser treatment[3]. Furthermore, the amount of anti-HA IgG measured by the laser-assisted assay was independent of the depth of microneedle penetration, eliminating potential variability based on how clinicians or individuals apply the MNA. The amount of biomarkers on the MNA would not be changed after 1 hr, substantially reducing false positives in case the MNAs are unintentionally left in the skin longer than necessary in the clinical setting.

4 Skin delivery of attenuated malaria sporozoite vaccines

Laser-induced permeability of capillaries under the skin may enhance not only leakage of circulating biomarkers, but also entrance of drugs or vaccines into circulation. One promising example is the facilitation of entry of radiation-attenuated sporozoites (RAS) into capillaries after intradermal (ID) injection. Malaria infects an estimated 207 million people every year worldwide, demanding more effective prevention and treatment [29]. These highly adaptable parasites are capable of developing drug and insecticide resistance, and vaccination strategies remain the most effective means to sustainably protect populations from malaria. There is evidence that protective immunity for malaria is mediated mainly by CD8⁺ T cells secreting interferon γ (IFN- γ) in the liver [30], so a promising experimental vaccine comprised of RAS that induces high CD8⁺ responses in the liver was tested in human volunteers [2]. While 100% of the subjects were protected sterilely, the treatment required six intravenous injections each at an interval of at least two weeks[30]. With such a dosing regimen, vaccinating healthcare workers and travelers would be plausible but immunization of large populations would be logistically problematic, because skilled providers are required for IV injections.

Live mosquitos with RAS in their salivary glands were discovered to confer malarial immunity decades ago, but using real mosquito bites to immunize large numbers of people would be impractical and perhaps unethical[31]. Unfortunately, attempts at intradermal or subcutaneous injections of the same vaccine have proven ineffective despite their apparent similarity to mosquito bites [30,32], because neither route delivers RAS directly into blood vessels so that they cannot reach the liver sufficiently, in contrast to IV injection[33,34]. It is currently not known how mosquito bites confer immunity with potency close to that of IV injections but ID injections cannot.

It is possible that mosquitoes injure vasculature with their proboscises during their probing for blood food via a vessel and then parasites deposited into the dermis during feeding enter circulation via these damaged vessels[35]. Unlike mosquito bites, ID injection doesn't damage blood vessels frequently and therefore does not confer the same degree of immunity as mosquito transmitted RAS. Considering that capillary injury may also be the key to delivery of malarial sporozoite vaccines into circulation, a similar solution was proposed for RAS delivery by Zhou, et al[4]. In this case, the purpose of increasing capillary permeability with lasers would be to allow entry of sporozoites deposited by ID injection (Figure 3) for immunization, rather than exit of biomarkers from circulation for detection.

In comparison with ID injection alone, illumination of the inoculation site with a 532nm pulse Nd:YAG laser, a duration 30s, an fluence 1J/cm² considerably improved ID delivery of sporozoites to the liver via circulation. It was shown that only about 10% of ID injected RAS reached the liver via circulation without laser treatment, but the liver-reaching rates elevated to 70% by laser treatment of the inoculation site[4], which was even greater than mosquito bites that delivered about 35% sporozoites into the circulation [36]. In agreement with the finding that higher counts of RAS in the liver resulted in stronger immunity, anti-sporozoite antibody titer was substantially higher in the presence as compared to absence of laser treatment. CD8⁺ IFN- γ ⁺ T cells in peripheral blood mononuclear cells (PBMCs) and in the liver were also significantly higher with laser pre-treatment than without, and raised to levels comparable to that measured after IV immunization[4]. Consequently, inoculation of RAS into the laser-illuminated skin elicited strong adaptive immunity and protected 88% mice from malarial infections, as opposed to 0% for ID injection alone under similar vaccination doses[4].

These promising results suggest that laser-mediated facilitation of skin-to-liver delivery of RAS could provide a practical alternative to IV immunization in the clinics. Ultimately, the potential in this technology lies in combining lasers with microneedle vaccine delivery. There is great promise in MNA enabled intradermal injection [37-39], allowing ID immunization without the aid of a skilled worker, so a small handheld device can be fabricated incorporating laser illumination and MNAs for efficient and convenient delivery of RAS vaccines or therapeutic drugs.

5 Conclusion

Despite the remarkable capacity for selectively heating skin capillaries with lasers, therapeutic utility of Hb photoexcitation has been limited to dermatology for several decades. It is now discovered that instead of rupture, lasers can be set to temporarily increase permeability of vessels, giving clinicians a tool for releasing biomarkers from or introducing drugs into circulation. As animal experiments have confirmed the concept's great potential, the techniques based on this novel concept are expected to develop in broad applications.

In the future, MNA-based biomarker detection systems could be designed to provide visual signals for smart phones fitted with micro lenses, allowing mobile analyses for at home monitoring and onsite diagnosis of diseases. Integration of laser illumination and MNAs into a single device may also be possible, using transparent materials to fabricate MNAs such that laser light could be transmitted through the MNAs themselves. Not only would these devices be portable, but also they would allow lasers to penetrate the skin past melanocytes independent on patients' skin types. By direct illumination of the dermis, as opposed to the skin surface, the laser treatment would grant a broad application ranging from blood biomarker detection to therapeutic drug delivery.

Acknowledgement

This work was supported by the National Institutes of Health grants AI089779, AI070785, and AI097696 (to M.X.W.).

References

1. Nir Y, Paz A, Sabo E, Potasman I. Am J Trop Med Hyg. 2003; 68:341–344. [PubMed: 12685642]
2. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, Holman LA, James ER, Billingsley PF, Gunasekera A, Richman A, Chakravarty S, Manoj A, Velmurugan S, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Plummer SH, Hendel CS, Novik L, Costner PJ, Mendoza FH, Saunders JG, Nason MC, Richardson JH, Murphy J, Davidson SA, Richie TL, Sedegah M, Sutamihardja A, Fahle GA, Lyke KE, Laurens MB, Roederer M, Tewari K, Epstein JE, Sim BK, Ledgerwood JE, Graham BS, Hoffman SL. Science. 2013; 341:1359–1365. [PubMed: 23929949]
3. Li B, Wang J, Yang SY, Zhou C, Wu MX. Biomaterials. 2015; 59:30–38. [PubMed: 25950985]
4. Zhou C, Chen X, Zhang Q, Wang J, Wu MX. J Control Release. 2015; 204:30–37. [PubMed: 25725360]
5. Anderson RR, Parrish JA. Science. 1983; 220:524–527. [PubMed: 6836297]
6. Chandrasekhar S, Iyer LK, Panchal JP, Topp EM, Cannon JB, Ranade VV. Expert Opin Drug Deliv. 2013; 10:1155–1170. [PubMed: 23662940]
7. El Laboudi A, Oliver NS, Cass A, Johnston D. Diabetes Technol Ther. 2013; 15:101–115. [PubMed: 23234256]
8. Birchall JC. Expert Rev Med Devices. 2006; 3:1–4. [PubMed: 16359245]
9. Prausnitz MR. Adv Drug Deliv Rev. 2004; 56:581–587. [PubMed: 15019747]
10. Chen X, Wang J, Shah D, Wu MX. Expert Rev Vaccines. 2013; 12:1313–1323. [PubMed: 24127871]
11. Chen X, Wu MX. Expert Rev Vaccines. 2011; 10:1397–1403. [PubMed: 21988305]
12. Houk LD, Humphreys T. Clin Dermatol. 2007; 25:434–442. [PubMed: 17870521]
13. Rubin IK, Farinelli WA, Doukas A, Anderson RR. Lasers Surg Med. 2012; 44:152–157. [PubMed: 22241659]
14. Anderson RR. J Dermatol. 2000; 27:700–705. [PubMed: 11138535]
15. Astner S, Anderson RR. Dermatol Ther. 2005; 18:267–281. [PubMed: 16229727]
16. Wall TL. Semin Plast Surg. 2007; 21:147–158. [PubMed: 20567666]
17. Vaught JB. Cancer Epidemiol Biomarkers Prev. 2006; 15:1582–1584. [PubMed: 16985016]
18. Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, Krueger KE, Rom W, Sanda M, Sorbara L, Stass S, Wang W, Brenner DE. J Proteome Res. 2009; 8:113–117. [PubMed: 19072545]
19. Bowen RA, Hortin GL, Csako G, Otanez OH, Remaley AT. Clin Biochem. 2010; 43:4–25. [PubMed: 19822139]
20. Anderson NL. Clin Chem. 2010; 56:177–185. [PubMed: 19884488]
21. Donovan DM, Bigelow GE, Brigham GS, Carroll KM, Cohen AJ, Gardin JG, Hamilton JA, Huestis MA, Hughes JR, Lindblad R, Marlatt GA, Preston KL, Selzer JA, Somoza EC, Wakim PG, Wells EA. Addiction. 2012; 107:694–708. [PubMed: 21781202]
22. Tripathi S, Prabhakar A, Kumar N, Singh SG, Agrawal A. Biomed Microdevices. 2013; 15:415–425. [PubMed: 23355067]
23. Zhang J, Wei X, Xue X, Jiang Z. J Nanosci Nanotechnol. 2014; 14:7419–7426. [PubMed: 25942803]
24. Bhargav A, Muller DA, Kendall MA, Corrie SR. ACS Appl Mater Interfaces. 2012; 4:2483–2489. [PubMed: 22404111]
25. Coffey JW, Corrie SR, Kendall MA. Biomaterials. 2013; 34:9572–9583. [PubMed: 24044999]
26. Corrie SR, Fernando GJ, Crichton ML, Brunck ME, Anderson CD, Kendall MA. Lab Chip. 2010; 10:2655–2658. [PubMed: 20820632]
27. Lee KT, Muller DA, Coffey JW, Robinson KJ, McCarthy JS, Kendall MA, Corrie SR. Anal Chem. 2014; 86:10474–10483. [PubMed: 25232916]
28. Ng KW, Lau WM, Williams AC. Drug Deliv Transl Res. 2015; 5:387–396. [PubMed: 25939431]
29. World Health Organization. World Malaria Report. 2013 12-11-2013. Ref Type: Generic.
30. Epstein JE, Tewari K, Lyke KE, Sim BK, Billingsley PF, Laurens MB, Gunasekera A, Chakravarty S, James ER, Sedegah M, Richman A, Velmurugan S, Reyes S, Li M, Tucker K, Ahumada A,

- Ruben AJ, Li T, Stafford R, Eappen AG, Tamminga C, Bennett JW, Ockenhouse CF, Murphy JR, Komisar J, Thomas N, Loyevsky M, Birkett A, Plowe CV, Loucq C, Edelman R, Richie TL, Seder RA, Hoffman SL. *Science*. 2011; 334:475–480. [PubMed: 21903775]
31. Clyde DF, Most H, McCarthy VC, Vanderberg JP. *Am J Med Sci*. 1973; 266:169–177. [PubMed: 4583408]
32. Conteh S, Chattopadhyay R, Anderson C, Hoffman SL. *PLoS One*. 2010; 5:e8947. [PubMed: 20126610]
33. Jin Y, Kebaier C, Vanderberg J. *Infect Immun*. 2007; 75:5532–5539. [PubMed: 17785479]
34. Yamauchi LM, Coppi A, Snounou G, Sinnis P. *Cell Microbiol*. 2007; 9:1215–1222. [PubMed: 17223931]
35. Matsuoka H, Yoshida S, Hirai M, Ishii A. *Parasitol Int*. 2002; 51:17–23. [PubMed: 11880224]
36. Amino R, Thiberge S, Martin B, Celli S, Shorte S, Frischknecht F, Menard R. *Nat Med*. 2006; 12:220–224. [PubMed: 16429144]
37. Wang J, Li B, Wu MX. *Proc Natl Acad Sci U S A*. 2015; 112:5005–5010. [PubMed: 25848020]
38. Norman JJ, Arya JM, McClain MA, Frew PM, Meltzer MI, Prausnitz MR. *Vaccine*. 2014; 32:1856–1862. [PubMed: 24530146]
39. Chen X, Kositratna G, Zhou C, Manstein D, Wu MX. *J Control Release*. 2014; 192:310–316. [PubMed: 25135790]

Figure 1.

Tracking FITC leakage by intravital laser confocal microscopy in laser-treated skin. Control skin illuminated by sham light displayed FITC confined within blood vessels, while FITC was extravasated from the vessels. Bar, 200 μ m. Reproduced from Ref.[3]

Figure 2.

Schematic illustration of laser treatment effect on plasma biomarker turnover. (A) The skin without laser treatment. (B) Circulating biomarkers leak from a capillary damaged by one of the MNs in the array, and bind to a MN nearby as seen in the right panel. (C) Laser-induced extravasation allows all MNs in the array to experience uniform biomarker exposure, leading to consistent binding on all MNs in the array (right panel). Reproduced from Ref.[3]

Figure 3.

Graphic representation of introducing RAS into circulation. A mosquito probes an area of skin, damaging blood vessels and depositing RAS. However, when RAS is injected by a needle, most sporozoites do not enter circulation. Laser illumination allows more RAS to enter capillaries by increased permeability of capillary. Reproduced from Ref.[4]