Hydrogel-Coated Microneedle Arrays for Minimally Invasive Sampling and Sensing of Specific Circulating Nucleic Acids from Skin Interstitial Fluid

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ABSTRACT: Minimally invasive technologies that can sample and detect cell-free nucleic acid biomarkers from liquid biopsies have recently emerged as clinically useful for early diagnosis of a broad range of pathologies, including cancer. Although blood has so far been the most commonly interrogated bodily fluid, skin interstitial fluid has been mostly overlooked despite containing the same broad variety of molecular biomarkers originating from cells and surrounding blood capillaries. Emerging technologies to sample this fluid in a pain-free and minimally-invasive manner often take the form of microneedle patches. Herein, we developed microneedles that are coated with an alginate−peptide nucleic acid hybrid material for sequence-specific sampling, isolation, and detection of nucleic acid biomarkers from skin interstitial fluid. Characterized by fast sampling kinetics and large sampling capacity (~6.5 μL in 2 min), this platform technology also enables the detection of specific nucleic acid biomarkers either on the patch itself or in solution after light-triggered release from the hydrogel. Considering the emergence of cell-free nucleic acids in bodily fluids as clinically informative biomarkers, platform technologies that can detect them in an automated and minimally invasive fashion have great potential for personalized diagnosis and longitudinal monitoring of patient-specific disease progression.

KEYWORDS: microneedle, hydrogel, skin interstitial fluid, microRNA, biomarker
validating this type of bodily fluid as a greatly overlooked source of biomarkers for personalized medicine. Surrounding cells within a tissue, ISF serves as an exchange medium between blood plasma and cells and contains a combination of molecular constituents found in both sources. Skin ISF is found within several hundred microns of the skin surface, primarily in the connective tissue dermis where only a few capillary beds and pain receptors reside. It can therefore be sampled in a pain-free manner, without any risk of blood contamination. This contrasts with blood drawing techniques that can be invasive (venous blood) or result in poor quality samples (fingerstick capillary blood).

Minimally invasive technologies for skin ISF sampling have emerged that are based on compact patches of microneedles (MNs). They are typically made of an array of microscale solid, porous, or hollow needles from materials such as glass, metal, silicon, or other polymers. Hollow needles were designed to create pathways for ISF extraction via capillary force or vacuum-induced suction. They represent very useful alternatives to invasive sampling technologies traditionally based on microdialysis and requiring tubing implantation under local anesthetics. Current limitations of many of the MN patches engineered so far include low sampling capacity (<2 μL) and/or long sampling times (e.g., >1 h to sample enough ISF volumes for subsequent biomarker analysis). In addition, to the best of our knowledge, there has been no report of MNs engineered to sample and detect specific nucleic acid biomarkers from skin ISF. So far, MNs were at best used for sampling and releasing total skin ISF and circulating nucleic acids detected after heavy sample processing and PCR-based analysis.

Herein, we report on the engineering of hydrogel-coated MN patches that can sample and isolate specific miRNA biomarkers from skin ISF at the fastest rate, while enabling the captured miRNA to be detected in situ (Figure 1a). Our versatile platform also offers the capability of light-triggered release of the miRNA for post-sampling off-chip analysis. Poly(i-lactide) (PLLA) arrays of 77 microneedles were chosen as our sampling platform, as we previously reported their successful use for either transdermal vaccine delivery or ISF/cell sampling from the skin.

RESULTS AND DISCUSSION

Arrays (7 × 7 mm²) were produced, as previously reported by us, that were decorated with pyramidal shaped MNs (Figure S1). The height of the needles was set to 550 μm to enable them to penetrate through the epidermis layer and reach the underlying ISF-containing dermis layer. For sampling and isolation of specific miRNA biomarkers from skin ISF, the MN array was coated with alginate–PNA hydrogel coated MNs that have been reported to be optimized for the extraction and immobilization of the target miRNA.

Figure 1. Schematic representation of the hydrogel-coated microneedle platform during sampling of the interstitial fluid. (a) Microneedle arrays (MN) are functionalized with bespoke peptide nucleic acid (PNA) probes (blue) which are covalently bound to an alginate hydrogel matrix via a photocleavable linker (PCL, yellow). Minimally invasive sampling of skin interstitial fluid can be achieved by pressing the coated MN patch onto the skin for 15 min. (b) Scanning electron micrograph (5.0 kV, 100X magnification, 10 nm gold sputter coating) of the bare MNs and alginate–PNA hydrogel coated MNs (scale bar = 100 μm). (c) Schematic illustration of the generic protocol for MN sampling of target biomarker (red) and purification to remove nontarget sequences (green). Circles represent a magnification of the alginate hydrogel coating on the MN patches. (i) When the MN is applied to sample a solution containing DNA, the target DNA sequence (red) hybridizes to the PNA probe (blue), forming a PNA:DNA duplex. (ii) MNs are washed to remove any nonspecific molecules (green) which have diffused into the hydrogel matrix. (d) Swelling kinetics of the hydrogel MNs fitted by the Spring and Dashpot Voight-based model (black solid line), showing an equilibrium swelling capacity of 6.5 ± 0.2 μL and a sampling rate constant of 0.74. Error bars show SEM (N = 6 MN patches).
and sequence-specificity when hybridizing to complementary DNA or RNA strands. Accessible through easily scalable solid-phase peptide synthesis, PNA's have proven to be highly valuable analytical tools for nucleic acid sensing, both in vitro and in vivo, and are particularly well suited for the detection of short oligonucleotides such as miRNAs. Herein, a 7-mer PNA (of sequence GACACGC) was designed that was complementary to the 5' end of miR-210 (5' CUGUCCGU-...).
GUGACAGCGGCUGA<sup>5′</sup>), a recently identified biomarker for early systemic melanoma recurrence, and chosen as a model system for our proof-of-concept study (Figure S2). The melanoma patients with abnormally elevated levels of circulating miR-210 were indeed found to be more likely to have disease recurrence, reinforcing the need for a noninvasive test suitable for longitudinal monitoring<sup>35,55</sup>. Throughout this proof-of-concept study, a DNA version of miR-210 (DNA-210, <sup>5′</sup>CTGTGCCTGTGACAGCGCCTGA<sup>3′</sup>) was chosen as our target of interest.

The PNA capture probe (Figure S2) was functionalized at its C-terminus with an alkyne moiety to facilitate its covalent immobilization to an azide-modified alginate via copper-catalyzed cycloaddition reaction (click chemistry). A photo-cleavable linker (3-amino-3-(2-nitrophenyl)propanoic acid) (PCL) was also introduced between the alkyne and the PNA sequence to enable the release of the PNA:DNA hybridization complex post ISF sampling via photoactivation with near-UV light (<360 nm) (Figure 1a). PNA length of 7-mer was chosen to allow stable PNA:DNA or PNA:RNA heteroduplex formation at human body temperature (melting temperature, <i>T_m</i> > 37 °C), as previously reported<sup>56</sup>.

The alginat−azide polymer was prepared as previously reported by EDC/NHS mediated peptide coupling between low-viscosity alginate and 11-azido-3,6,9-tri oxyundecan-1-amine (Figure S3), leading to an average level of azide functionalization of 17 mol% (Figure S5).<sup>57</sup> The alginat−PNA hybrid material was finally assembled by an azide−alkyne cycloaddition reaction, in the presence of Cu(II) sulfate, Tris/(1-benzyl-1H-1,2,3-triazol-4-yl)methylamine (TBTA), and sodium ascorbate (Figure S4), leading to an overall level of PNA functionalization of 1 mol% as assessed by 1H NMR spectroscopy (Figure S5).

Coating of the MN arrays with the newly engineered alginat−PNA proceeded in three steps: precoating with poly(1-lysine), followed by deposition of the alginate, and finally physical cross-linking with calcium chloride (CaCl<sub>2</sub>), leaving enough time for the MN to dry between each step. Once fully dried, scanning electron microscopy (SEM) was used to characterize and compare the physical morphology at the surface of the MN patches with and without alginate−PNA hydrogel coating (Figure 1b). To determine the effect of PNA functionalization on the alginate’s physical properties, MN patches coated with unmodified alginate were also analyzed. The SEM micrographs of both types of alginate displayed an interconnected network of pores with a relatively consistent pore size. Although SEM only provides information on the hydrogels’ structures in their nonswollen dehydrated form, it is noteworthy that the average pore size of the dehydrated alginat−PNA coating, however, was approximately half that of the unmodified alginate (Figure S6). This could be due to the hydrophobic nature of these charge-free PNAs limiting water uptake and reducing swelling, as previously observed when functionalizing hydrogel fibers with hydrophobic moieties.<sup>58</sup> Atomic force microscopy (AFM) was also used to gain an insight into the topography of the alginate−PNA on the surface. For ease of imaging, however, the hydrogel was deposited on a glass slide, but this time no lyophilization or metal coating was needed, therefore providing a more accurate representation of the hydrogel structure. The surface topography showed a relatively consistent and homogeneously distributed porous structure over the 50 × 50 μm<sup>2</sup> area with pores or voids of 200−800 nm (Figure S7), only slightly larger than those observed, after lyophilization, by SEM. As earlier studies from our group showed, small oligonucleotides the size of miRNAs could easily diffuse within such porous materials and hybridize to pre-embedded PNAs.<sup>59</sup>

The main limitations of existing ISF sampling platforms are their low sampling capacity and low sampling rates. For example, microdialysis techniques typically sample at 1−5 μL/min, while less invasive capillary ultrafiltration is even slower, at 100−150 nL/min. The swelling behavior of our hydrogel-coated MNs was assessed in buffer (PBS) and at physiological body temperature (37 °C). Figure 1d describes the volume of liquid absorbed by the MNs over time which can be fitted by the Spring and Dashpot Voight-based model commonly used for describing swelling kinetics of hydrogels. According to this model, the hydrogel-coated MNs have an equilibrium swelling capacity of 6.5 ± 0.2 μL, with a sampling rate constant of 0.74, meaning that 63% of the full swelling capacity is achieved in less than 1 min. This compares very favorably with other recently reported hydrogel-coated MN sampling technologies and can at least in part be attributed to the large surface area of the MNs due their pyramidal shape and porous coating structure.

To test the ability of our MN patches to sample and isolate nucleic acids in a sequence-specific manner, MNs were dipped into solutions (100 μL) containing various amounts of DNA-210 (0−500 nM) labeled with Alexa 647 dye. After 15 min sampling, the MNs were washed thoroughly with water and dried overnight at room temperature before imaging with a fluorescence scanner (Typhoon FLA9500, GE Healthcare). As shown in Figure 2a, a plot of the mean fluorescence intensity (N = 22 individual microneedles from 2 different MN patches) versus DNA concentration demonstrates the ability of our patches to detect target concentrations as low as ~6 nM, with a linear regime across almost 2 orders of magnitude (6−500 nM). Sequence specificity was then confirmed by demonstrating the statistically significant ability of the MN patch to discriminate between a complementary and a noncomplementary DNA target, both labeled with the same fluorophore (Figure 2b).

In order to demonstrate the possibility of releasing the captured nucleic acid from the microneedle, MN patches preincubated with fluorescently labeled DNA-210 were placed tips-down in water (100 μL) within a UV cross-linker (UVP) and irradiated with increasing amounts of UV energy (λ<sub>ex</sub> = 315 nm, 0−4 J/cm<sup>2</sup>). After shaking for 1 h, the MNs were rinsed, dried, and imaged on a fluorescence scanner. A significant loss in fluorescence intensity of the MN was observed post-irradiation that suggested the release of over 70% of the captured DNA after 1 min of irradiation (Figure S8).

Our MN patches were designed not only to sample specific endogenous nucleic acid biomarkers from skin ISF, but also to enable their quantitative detection once sampled. Two different mechanisms for sensing were explored that involved either (i) the direct visualization of the isolated biomarker while captured on the microneedle patch or (ii) an alternative two-step process involving light-triggered release of the PNA:DNA complex followed by detection in solution (Figure 2c). For both sensing strategies, the MN patches were initially dipped into solutions (100 μL) containing various amounts of unlabeled DNA-210 (10−200 nM), then washed thoroughly to remove any unbound DNA and dried. For direct visualization, the MN patches were then incubated in a solution of DNA...
intercalator (SYBR Safe, 2× concentration, Invitrogen), washed, and imaged with a fluorescence scanner (Figure 2d). For indirect visualization, the DNA-loaded MNs were then placed tips-down into 100 μL of water and photoradiated for 3 min at 3 J/cm² in a photo-cross-linker (BLX-315, λex = 315 nm). A solution of DNA intercalator was then added to detect the PNA:DNA duplex released in solution (Figure 2e). Both strategies proved successful at detecting nM concentrations of nucleic acids sampled with our MN patches, highlighting the versatility of this platform. While simpler and more direct on-chip detection is perfectly suited for applications that require testing at the point-of-care, the possibility of releasing the captured and purified (i.e., separated from all other ISF constituents, including other nucleic acid) material offers the option to detect and sequence less abundant biomarkers (through amplification-based methodologies). It is noteworthy, however, that both detection approaches are mutually complementary and could even potentially be carried out sequentially.

Having validated the sensitivity and selectivity of the MN sampling in vitro, we sought to investigate the sampling of specific nucleic acids from skin ISF directly in human skin, using an ex vivo model. Human abdominal skin samples were first prepared by incubation with either a complementary (DNA-210) or a noncomplementary (DNA-141) oligonucleotide labeled with Alexa-647 dye (500 nM each) and then washed thoroughly with water. MN patches were then pressed onto the skin surface (15 min, 37 °C) for sampling and then washed extensively and dried overnight before fluorescence imaging (Figure 3a). Images were analyzed by taking the average fluorescence of individual microneedles on each patch (N = 48 microneedles from three different MN patches). These results demonstrate not only that our MNs can indeed sample nucleic acids from skin ISF, but also that they retain their high sequence specificity, capturing preferentially (15-fold) the DNA fragment complementary to the PNA incorporated into the hydrogel (Figure 3b).

To confirm these findings, a second experiment was prepared where the skin samples were incubated in a solution containing a mixture of both DNA-210 (red bars) and DNA-141 (green bars) labeled with Alexa-647 and 6-FAM, respectively. After sampling (as described above), MN patches (N = 3 per condition) were imaged successively under two excitation filters (Figure 3c). While no significant difference between the experiments with and without DNA was detectable with the Cy2 filter (for 6-FAM visualization), a very strong signal increase was observed between the DNA-free control and the experiment with DNA with the Cy5 filter (for Alexa-647 visualization), confirming the efficient and sequence-specific capture of DNA-210 spiked within human skin ISF. Fluorescent confocal imaging of the MNs was also performed to confirm the previous findings and visualize the DNA captured around each MN (Figure 3d). Noncomplementary DNA-141 imaged with 488ex/510em showed little to no fluorescence signal on the MNs (Figure 3d, left), while complementary DNA-210 imaged with 647ex/665em showed fluorescence signal bound to the MN (Figure 3d, middle). A 3D projection of the fluorescence signal from a single microneedle is also shown (Figure 3d, right).

CONCLUSION

In summary, we have developed MN patches coated with hybrid alginate–PNA hydrogels that can sample up to 6.5 μL of fluid in 2 min. Unlike other sampling technologies reported to date, we demonstrated that attaching PNA oligomers to the hydrogel’s fibers also enables the specific sampling, purifica-
tion, and release of the only nucleic acid fragments that are complementary to the PNA sequence. This versatile platform can therefore be easily tuned by simply adapting the PNA sequence to that of any miRNA of interest. Functionalization of the hydrogel with different PNA sequences complementary to different miRNAs is also currently underway in our laboratories, which will enable the sampling and sensing of multiple miRNAs simultaneously (known as multiplexed analysis or profiling). Optical sensing of the captured biomarkers is also possible, either directly on-chip or in-solution after an additional light-triggered release step. Using a human skin ex vivo model, we also demonstrated that this technology could efficiently capture nucleic acids spiked within skin interstitial fluids with both high efficiency and sequence specificity. With the recent experimental evidence that skin ISF contains the same RNA species (including circulating miRNAs) as blood with comparable natural abundance, minimally invasive technologies that can not only sample this body fluid but can also interrogate its composition have the potential to transform the field of molecular diagnostics from liquid biopsies.

EXPERIMENTAL METHODS

Solid Phase PNA Synthesis. PNA probe directed against miR-210 was designed to contain an alkyl group (C-terminus) for ease of attachment to the alginate hydrogel fibers as well as a photosensitive group for ease of release by UV irradiation after sampling. The 7-mer PNA oligomer (Figure S2) was synthesized via standard solid phase peptide synthesis (SPPS) exploiting the chemistry of 9-fluoremethoxycarbonyl (Fmoc) protecting groups.

Synthesis of Alginate–PNA Conjugates. Alginate was functionalized with azide groups via peptide bond formation following a protocol adapted from Breger et al. (Figure S3). Briefly, a 1 wt % solution of alginate was prepared by dissolving 500 mg of alginate (low viscosity alginate from brown algae, ∼100 000 g/mol Sigma) in 50 mL of MES buffer (50 mM, pH 4.0). To this solution, the following was added sequentially: 20 mM N-ethyl-N’-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl, Sigma), 140 mM N-hydroxysuccinimide (NHS, Sigma), and 1.8 mL of 11-azido-3,6,9-trioxaundecan-1-amine (AA, SelectLab 134179-38-7). The reaction was set at RT overnight with constant stirring. The reaction mixture was then dialyzed (MWCO 12 kDa) against aqueous NaCl for 1 day, then against ddH2O for 3 days. Finally, the purified product was lyophilized to produce a white product, which was characterized by 1H NMR (D2O, 400 MHz, 363 K).

Alginate-azide was functionalized with PNA–alkyne via a copper-catalyzed azide–alkyne cycloaddition reaction (Click chemistry) as adapted from Presolski et al. Briefly, a 1 wt % solution of alginate–azide was prepared in 100 mM phosphate buffer (pH = 7.4). To this solution, PNA–alkyne was added at an amount equivalent to 1 mol % (i.e., 8.1 mg PNA–alkyne per 100 mg alginate–azide). Next, copper(II) sulfate (CuSO4, Sigma) and the ligand Tris[1-(benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTAt, Sigma) were mixed together, then added to the reaction solution to produce a final concentration of 0.1 mM CuSO4 and 0.5 mM TBTAt. Finally, sodium ascorbate (Sigma) was added to the solution at a final concentration of 5 mM. The reaction vessel was then sealed and left to react at RT overnight. After 24 h, the reaction solution was diluted 5 times, and the reaction was left to proceed for a further 24 h. To chelate and remove copper(II) ions from the solution, 10 mM ethyleneediamine tetraacetic acid (EDTA, Sigma) was added before the entire solution was dialyzed against ddH2O for 3 days, lyophilized, and characterized by 1H NMR (D2O, 400 MHz, 363 K).

Preparation of Hydrogel-Coated Microneedles. Poly(l-lactide) (PLLA; RESOMER L 207 S, Evonik Industries AG) MNs were prepared as previously reported by us. The dimensions of the MN patch can be seen in Figure S1. It is noteworthy that the height of the MN patch, which is 0.55 mm or 500 μm, was designed to allow the needles to penetrate the epidermis layer (100–200 μm) and reach the underlying dermis layer, containing a rich source of ISF. The bare MNs were functionalized with an engineered hydrogel coating via a three-step coating procedure based on a protocol developed by Mandal et al. First, 50 μL of a 0.01 wt % solution of positively charged poly(l-lysine) (150 000–300 000 g/mol Sigma, P8432) was pipetted onto each MN to form an adsorbed layer that will subsequently facilitate electrostatic adhesion of the alginate hydrogel. The solution was removed after 30 min, and the MNs were left to dry under a fume hood at RT for at least 1 h. Second, 60 μL of alginate solution, composed of 0.35 mg alginate–PNA and 1.4 mg sucrose (Sigma) in 60 μL ddH2O, was pipetted onto each MN array. The MNs were then left to dry under a fume hood at RT for at least 3 h. Finally, 50 μL of cross-linking solution, composed of 20 mM CaCl2 (Sigma), was pipetted onto each MN, after which the coated arrays were left to dry at RT overnight (>12 h).

Sampling Protocol. To visualize the captured DNA after MN sampling, fluorescently labeled (Alexa-647, unless otherwise indicated) single-stranded target DNA-210 (as a proxy for miRNA-210) and non-target DNA-141 (as a proxy for miR-141) were purchased from Invitrogen. For sampling, single MN patches were placed tips-down into individual wells of a clear 48-well microplate (Corning, half area) filled with 100 μL of analyte solution. The solution consisted of either water/buffer (control), target DNA-210, or non-target DNA-141 at concentrations indicated for each experiment. After sampling at 37 °C for 15 min (unless otherwise indicated), the MNs were removed, washed thoroughly with ddH2O (10 min, 3 washes), and then imaged by a fluorescence scanner (Typhoon FLA 9500, PMT 300 V, 25 μm pixel resolution). In the case where DNA was labeled with Alexa-647 dye, the MNs were imaged after release to show the loss of fluorescence, equivalent to release of DNA (Typhoon FLA 9500, PMT 300 V, 25 μm pixel resolution). In the case where DNA was unlabeled, thiazole orange (TO, 2 μm) was added to the solution in each well and kept for 30 min before imaging with a plate reader (Omega, λex = 488 nm, λem = 520 nm, gain = 1000).

Preparation of Human Skin Samples. Human abdominal skin with adipose tissue was purchased from Caltag medsystems (Buckingham, U.K.). The sample was washed in Dulbecco’s minimal essential medium (DMEM; Gibco Life Technologies) supplemented with 2% Antimycotic-Antibiotic (ABAM; Gibco Life Technologies) for 30 min. Then, it was moved to DMEM supplemented with 1% ABAM for the rest of the procedure. Using sterile surgical scissors, subcutaneous fat was removed in order to obtain only the epidermis with the dermis. A series of 8 mm2 area punches were made using a biopsy punch (Stiefel) to create nine skin samples (N = 3 replicates per condition) for the following MN sampling experiment.

MN Application to Human Skin. MNs were pressed onto human skin samples by a gentle thumb press. After 15 min at 37 °C, MNs were gently removed. To show penetration, skin was stained with trypan blue (0.4% diluted in half by ddH2O, sterile-filtered, Sigma, T8154). After 10 min, skin samples were rinsed thoroughly then imaged by a wide field microscope under bright field illumination to show a characteristic MN penetration pattern.

MN Sampling from Skin. Just before sampling with MNs, skin samples were removed from the culture media (DMEM with 1% ABAM), then washed thoroughly with ddH2O (three times). To load the skin with DNA, the samples were gently transferred to 48 wells microlautes and placed on top of 100 μL solutions containing ddH2O
(control), 500 nM nontarget DNA-141, or 500 nM target DNA-210, where both DNA fragments were labeled with Alexa-647 dye (N = 3 samples per condition). Skin samples were left to incubate in the solutions overnight in the fridge. On the next day, the samples were carefully removed from the incubation solutions with sterile tweezers, rinsed thoroughly with ddH$_2$O, then placed in a 48-well microplate. For the MN sampling experiment, single MN patches were gently pressed onto each skin sample by a gentle thumb press, and the MNs were left to sample at 37 °C for 15 min. Next, the MNs were gently removed from the skin, rinsed thoroughly (ddH$_2$O, three times), and left to dry overnight before being imaged with a fluorescence scanner (Typhoon FLA 9500, PMT 400 V, 25 μm pixel resolution, λ$_{ex}$ = 635 nm, Cy5 setting). A second experiment was conducted exactly as the first described above but wherein skin samples were left to incubate in a solution containing a mixture of DNA:target DNA-210 tagged with Alexa-647 and nontarget DNA-141 tagged with fluorescein, both at 500 nM. The rest of the experiment was prepared as outlined above, but the MNs were imaged after sampling at two wavelengths: 635 nm (Cy5 setting) to image the target DNA-210 and 473 nm (Cy2 setting) to image the nontarget DNA-141.

It is important to note that when incubating the skin samples in the solutions containing fluorescently labeled DNA, the samples floated on the surface with the bottom dermis layer of the skin in contact with the solution. Molecules from the solution could not cross the intact and highly impermeable stratum corneum from the top. Thus, after sampling the skin with MNs, any fluorescence signal detected on the MN originated from molecules which have diffused from the solution through the dermis and into the epidermis.

ASSOCIATED CONTENT

3 Supporting Information

Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.9b04783.

Experimental procedures for the chemical and physical characterization of the PNA and PNA-alginate hydrogels (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES


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