Salivary-based bone loss marker detection platform for point-of-care screening
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INTRODUCTION
Bone health is regulated in a tightly coupled metabolic process between bone formation (by osteoblasts) and bone resorption (by osteoclasts). In healthy bone these processes are in balance; however, these rates may become uncoupled due to diseases affecting this regulation (Paget's disease, metastatic bone cancer), or hormonal changes, as in postmenopausal women. When bone resorption occurs more than bone formation, a net loss in bone mineral density (BMD) results, which can lead to diseases such as osteoporosis. The traditional approach to measuring BMD is dual energy X-ray absorption (DEXA), however DEXA is an expensive procedure not readily suited for general population screening. Alternatively, biomarkers of bone formation and degradation can be assayed in human serum or urine via concentrations of osteocalcin (OC) and deoxypyridinoline (Dpd), respectively, identifying the concentrations of these biomarkers in human saliva may lead to better diagnosis and prevention of osteoporosis, as well as offering a noninvasive method for convenient population screening. Here we report recent developments in a lateral flow test strip (LFTS) platform to measure OC and Dpd in saliva to identify early indications of bone loss and minimize bone fracture risk associated with osteoporosis.

METHODS

The LFTS platform is a rapid immunochromatographic assay comprised of a test strip with several membranes that house all the reagents necessary for the test. The analyst of interest is applied in the sample membrane (OC or Dpd in saliva), wherein it is captured in the test in a sandwich-antibody immunocomplex coupled to fluorescent detection. Monoclonal antibodies specific for OC or Dpd are conjugated to fluorescently labeled microparticles and deposited on the conjugate pad. Upon adding the sample to the sample pad, the saliva resolubilizes the dried antibody conjugates and forms an analyte-antibody conjugate complex, which is captured by another monoclonal antibody specific for OC or Dpd immobilized to the nitrocellulose membrane. Monoclonal antibodies were chosen to achieve the specificity needed for the assay.

RESULTS

Whole, unstimulated saliva samples from 20 donor patients were obtained in collaboration with the University of Mississippi Medical Center in Jackson, MS. Saliva samples were kept frozen at -80°C until tested. Testing Protocols:
1) Centrifuge samples to remove large particulates.
2) Dilute samples 1:1 with our running buffer.
3) Add 100 µL, sample volume, for each test. Each sample was assayed in triplicate.
4) Run test for 10 minutes, measure results with ESE reader.

CONCLUSIONS
1) A lateral flow assay platform was developed to detect clinically relevant concentrations of osteocalcin in saliva.
2) Salivary osteocalcin levels showed correlation with BMD values corresponding to clinical bone status.
3) A readout system that can be easily integrated for point-of-care (POC) applications was developed.
4) Future applications of this technology may help to better control and diagnosis of osteoporosis, thereby reducing the number of bone fractures and other risks associated with the debilitating loss of bone mineral density in the aging population.