Salivary-based bone loss marker detection platform for point-of-care screening

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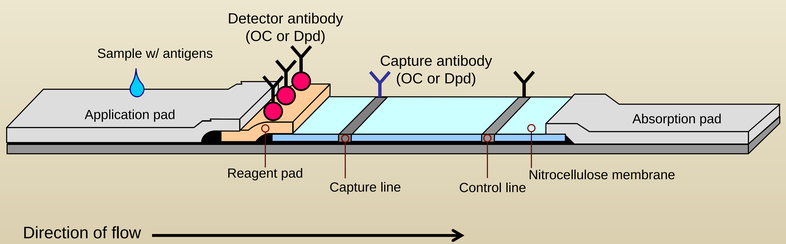
INTRODUCTION

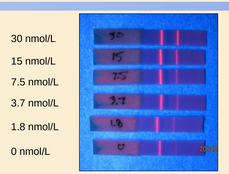
Bone health is regulated in a tightly coupled metabolic process between bone formation (by osteoblasts) and bone resorption (by osteoclasts). In healthy bone these processes are in balance; however, these rates may become uncoupled due to diseases affecting this regulation (Paget's disease, metastatic 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.

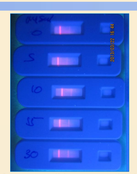
METHODS

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 analyte of interest is applied in the sample medium (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.





Formation of sandwich antibody detection with decreasing concentrations of **Dpd**. Fluorescence observed under UV light source.



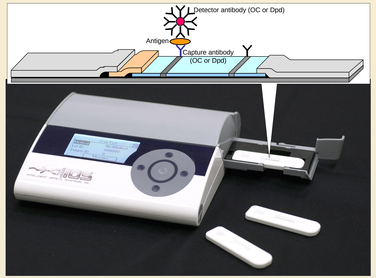
Intensity observed from spiking synthetic **OC** in saliva. Tests run in cassettes. Fluorescence observed under UV light source.

PROCEDURE

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.

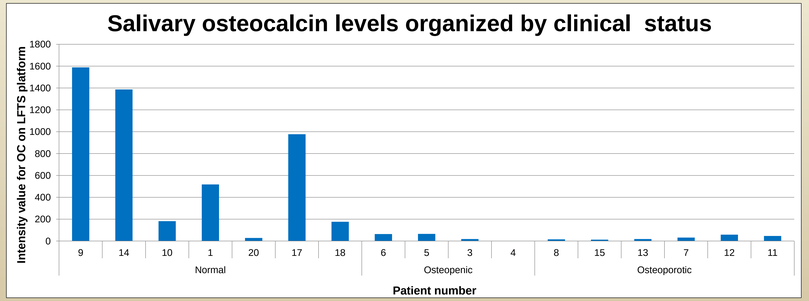
Testing Protocol:

1) Centrifuge samples to remove large particulates.
2) Dilute samples 1 to 1 with our running buffer.
3) Add 100 uL sample volume for each test. Each sample was assayed in triplicate.
4) Run test for 10 minutes, measure results with ESE reader.



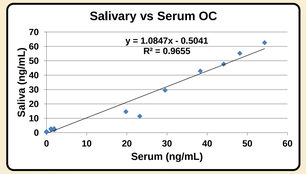
Separate, finalized test strips for OC and Dpd were placed in plastic cassettes for testing. The cassettes have an open window to view the test results, and a sample port where the sample is applied. We adapted the widely-used Qiagen ESE test strip fluorescent reader with customized optical settings of emission and excitation wavelengths to read the selected fluorescent label.

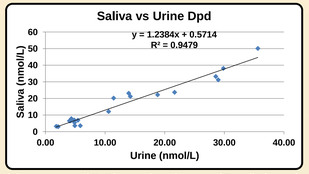
RESULTS



Patient samples categorized into three main groups – normal, osteopenic, and osteoporotic – showed high amounts of OC in normal patients, and low OC in osteopenic/osteoporotic patients.

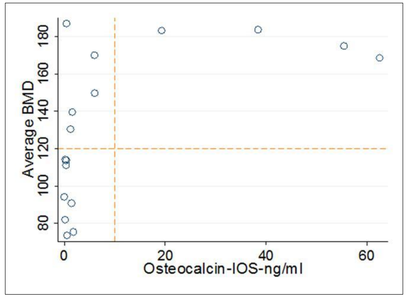
Correlating Salivary Biomarkers to Serum and Urine

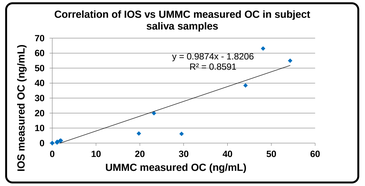




Salivary OC and Dpd concentrations were correlated with serum (OC) and urinary (Dpd) levels from the same patient using ELISA (Quidel). Samples were normalized by protein concentration to adjust for salivary specific gravity. The resulting high correlation suggested the reliability of salivary markers.

Performance of LFTS Platform Measuring Osteocalcin in Saliva





Quidel commercial ELISA test kit was used to validate the LFTS platform with patient saliva samples. A correlation value of 0.85 was obtained with OC.

Test Result	Non-Osteoporotic (BMD ≥ 120)	Osteoporotic (BMD < 120)
- Test (OC ≥ 10)	4 (44%)	0 (0%)
+ Test (OC < 10)	5 (56%)	8 (100%)
Total	9 (100%)	8 (100%)

Salivary OC levels compared against BMD with a cutoff concentration of 10 ng/mL of OC. There were zero false negatives (osteoporotic individual showing high OC levels) out of twenty patient samples.

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 patient 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 lead 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.