Proof-of-Concept for Salivary COMP Detection for Early Osteoarthritis Using an AI-driven Nano-engineered Biosensor

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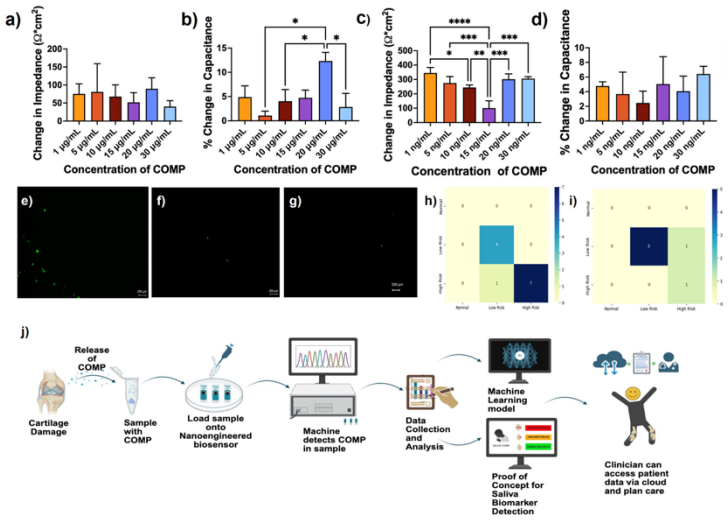
INTRODUCTION: Osteoarthritis (OA) is a leading cause of pain and disability worldwide, disproportionately affecting rural and underserved populations where access to orthopedic specialists and advanced diagnostic tools is limited.[1] In these communities, OA care is often managed by general practitioners. Yet, evidence-based strategies such as lifestyle modification and early intervention are inconsistently implemented due to time constraints, gaps in patient education, and limited knowledge specific to OA.[1] Many older adults perceive OA as a "normal part of aging," causing them to delay care until advanced joint damage has occurred. This is compounded in rural areas, which tend to have older populations, higher rates of obesity and physical inactivity, and significant barriers to specialty care, all of which worsen the outcome of OA.[2] Earlier diagnosis could enable timely, targeted interventions that slow disease progression, reduce pain, and preserve mobility, which is critical for patients with limited access to follow-up care. Current diagnostics, such as X-rays, detect OA only after irreversible cartilage loss is present.[3] While OA biomarkers have been studied in serum and synovial fluid, saliva remains underexplored despite being inexpensive, easily obtainable, and non-invasive. Recently, Cartilage Oligomeric Matrix Protein (COMP), a non-collagenous matrix protein associated with cartilage turnover, has been identified as a promising biomarker that has been measured in human serum and equine saliva, but not in human saliva.[4] Electrochemical biosensors have been a focused field of interest in diagnostics. This study presents the proof-of-concept for detecting COMP in saliva using a nano-engineered electrochemical biosensor. We hypothesize that the nano-engineered biosensor will aid in the early detection of OA using COMP. By comparing detection in artificial saliva and serum, we aim to determine whether saliva can serve as a reliable surrogate for systemic biomarker levels, laying the groundwork for a potential portable, rapid, low-cost point-of-care test for early OA diagnosis in rural and under-resourced communities.

METHODS: 1) Biosensor preparation and calibration: An electrochemical biosensor consisting of a gold working electrode was modified into a nano-engineered electrochemical biosensor using graphene oxide to increase the surface area and sensitivity. To determine optimal antibody concentration, different concentrations of COMP antibodies, such as 0.25, 0.5, 1.0, 1.25, and 1.5 µg/mL, were immobilized on the working electrode. DSP was used as a cross-linker to enhance antigen-antibody binding. Electrochemical impedance spectroscopy (EIS) data were obtained. Different electrochemical parameters were calculated from the EIS data to measure the change in impedance, percent change in capacitance, change in resistance (Rp), and cyclic voltammetry (CV). Next, a protein calibration curve was generated using different COMP concentrations, representative of clinical OA risk based on patient serum data. Based on a review of the literature, serum levels of COMP in OA patients were categorized as normal (1–5 µg/mL), low risk (10–15 µg/mL), and high risk (20+ µg/mL). A saliva calibration curve was also established from literature using a 1:1000 serum-to-saliva ratio: normal (1–5 ng/mL), low risk (10–15 ng/mL), and high risk (20+ ng/mL). **2) Biological Validation:** Antibody–antigen binding was confirmed qualitatively with confocal microscopy. To validate the accuracy of the nano-engineered biosensor, human fibroblast-like synoviocytes (HFLS) were first treated with varying concentrations of lipopolysaccharide (LPS) and lipoteichoic acid (LTA) for 72 hours and 24 hours, respectively, to mimic an inflammatory OA environment. The supernatants were then tested using confocal microscopy and ELISA. Blinded saliva samples with unknown COMP concentrations were also tested. Quantitative validation was performed with gold standard ELISA to determine COMP levels in LPS and LTA-treated HFLS supernatants. The results obtained from these biological assays were then compared to biosensor data. **3) Machine Learning Model:** EIS data collected from the biosensors were used to train a support vector machine (SVM) model and to classify OA risk based on COMP protein concentration levels.

RESULTS: The optimal antibody concentration determined for COMP was 1 µg/mL. EIS data were obtained for different protein concentrations, and electrochemical parameters were calculated in both serum and saliva. In serum, a common decreasing followed by an increase trend was seen in regards to the percent change in capacitance (Figure b.) and CV area with an increase in concentrations. The change in resistance and the change in impedance showed a varying trend (Figure a). In saliva, a common decreasing trend followed by an increasing trend was observed in regards to the change in impedance (Figure c), percent change in capacitance (Figure d), and CV area with an increase in concentration. The change in resistance showed a varying trend. Confocal microscopy of antigen-antibody binding revealed an increase in fluorescence with increasing protein concentrations of both serum and saliva. (Figure e). Both LPS and LTA-treated HFLS supernatants showed high fluorescence at higher concentrations of 100 ng/mL (Figure f.) and 500 ng/mL (Figure g.), respectively. A supervised ML model was run for the electrochemical parameters data, and the accuracy was found to be 68.18% in serum samples (Figure h.) and 72.73% for saliva samples (Figure i).

DISCUSSION: This study demonstrates the possibility of using a nano-engineered electrochemical biosensor to detect COMP in saliva, representing the first step toward a non-invasive, point-of-care diagnostic for early OA. In other clinical pathologies, saliva has shown promising biomarker data. It has been used for non-invasive cancer detection, such as identifying markers for pancreatic cancer with high sensitivity and specificity[5], oral cancer diagnostics using circulating tumor DNA and miRNAs in saliva[6], and cardiovascular risk assessment, via salivary C-reactive protein and other indicators.[7] These successes show that salivary biomarkers can accurately reflect systemic disease, reinforcing the translational promise of our work with COMP. By using both saliva and serum measurements, this project shows whether salivary COMP can act as a reliable surrogate for systemic OA biomarkers. The integration of machine learning could further improve diagnostic accuracy. Given the diagnostic delays faced by rural and underserved populations, a portable, low-cost screening tool could enable providers to identify OA earlier, initiate evidence-based interventions, and potentially alter the disease trajectory, especially for patients who might otherwise present only at the stage of surgical candidacy.



SIGNIFICANCE: OA is a major cause of disability, particularly in rural and underserved populations where delayed diagnosis worsens outcomes. This first-of-its-kind salivary COMP biosensor has the potential to deliver rapid, affordable, and non-invasive OA screening directly to primary care and community health settings, allowing earlier diagnosis, intervention, and improved long-term patient outcomes.

REFERENCES: [1]Ali et al. 2018 [2]Befort et al., 2012 [3]Guermazi et al., 2009 [4]Tseng et al., 2009 [5]Zhang et al., 2016 [6]Cristaldi et al., 2019; [7]Bahbah et al., 2021.

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Figure 1: a) Graph of change in impedance vs different concentrations of artificial serum. b) Graph of % change in capacitance vs different concentrations of COMP in artificial serum. c) Graph of change in impedance vs different concentrations of COMP in artificial saliva. d) Graph of % change in capacitance vs different concentrations of COMP in artificial saliva. e) Confocal imaging of 30 µg/ml COMP in artificial serum. f) Confocal imaging of HFLS treated with 100 µg/ml of LPS. g) Confocal imaging of HFLS treated with 500 ng/ml of LTA. h) SVM model of data from different COMP concentrations in artificial serum with the accuracy of 68.18%. i) SVM model of data from different COMP concentrations in artificial saliva with an accuracy of 72.73%. j) Overview of the project.