CSAC 2021

THE 1ST INTERNATIONAL ELECTRONIC CONFERENCE ON CHEMICAL SENSORS AND ANALYTICAL CHEMISTRY

ELECTROCHEMICAL IMMUNOSENSOR FOR SIMULTANEOUS DETERMINATION OF EMERGING AUTOIMMUNE DISEASE BIOMARKERS IN HUMAN SERUM

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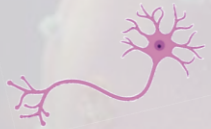
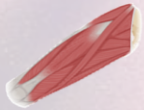
AUTOIMMUNE DISEASES

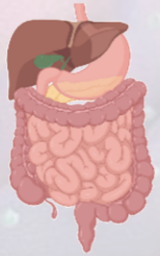


NERVES
peripheral neuropathy
diabetic neuropathy

LUNG
fibromyalgia
Wegener's granulomatosis

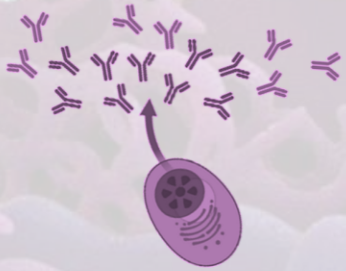


MUSCLES
muscular dystrophy
fibromyalgia



GASTROINTESTINAL TRACK
celiac's disease
Crohn's disease
ulcerative colitis
type I diabetes

SKIN
psoriasis
vitiligo
eczema
scleroderma



AUTOIMMUNE DISEASES

THYROID
thyroiditis
Hashimoto's disease
Graves' disease

BLOOD
leukemia
lupus erythematosus
hemolytic dysglycemia

BRAIN
multiple sclerosis
Guillain-Barre syndrome
autism

BONES
rheumatoid arthritis
ankylosing spondylitis
polymyalgia rheumatica

POSSIBLE CAUSES AND RISK FACTORS

AGE
symptoms usually begin between 15 and 45 years

GENDER
affects women more than men

LIFESTYLE
western diet, alcohol and tobacco

HEREDITY
genetic predisposition

ENVIRONMENT
chemicals, heavy metals, infections, stress

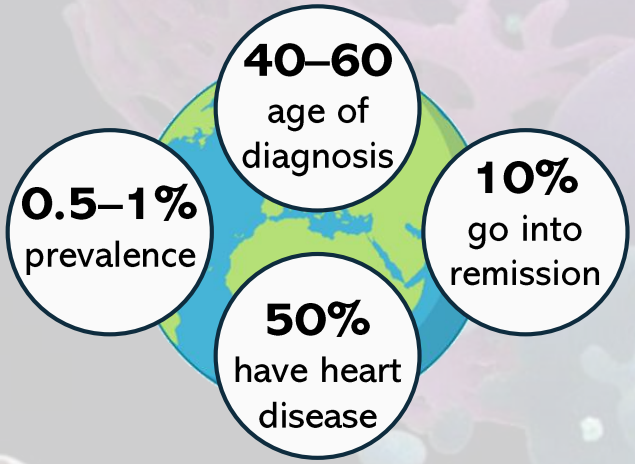
HYGIENE HYPOTHESIS
vaccines, antiseptics

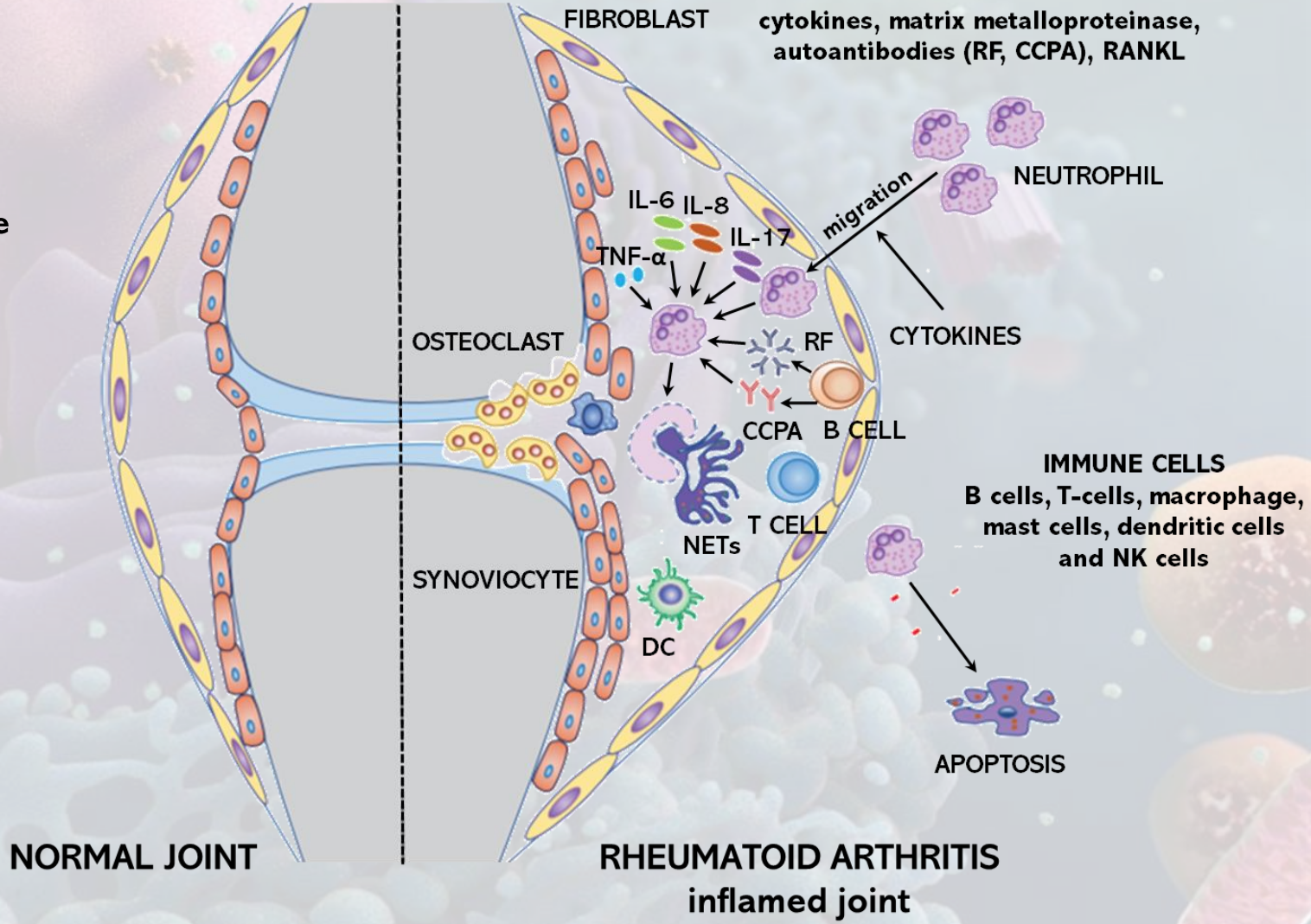
RHEUMATOID ARTHRITIS

1. characterized by joint inflammation and destruction
2. functional limitations
3. working disability
4. poor quality of life

ATTENTION

EARLY DIAGNOSIS AND TREATMENT REDUCE JOINT DESTRUCTION, PRESERVE FUNCTIONALITY AND INCREASE SURVIVAL RATE



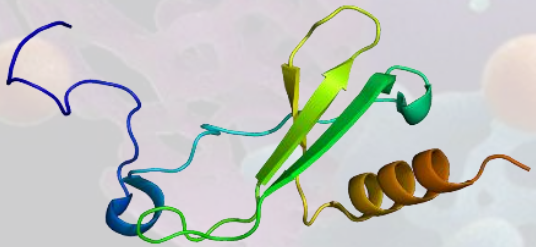


RHEUMATOID ARTHRITIS BIOMARKERS

NEUTROPHIL ACTIVATING PROTEIN-2 (CXCL7)

highly expressed in serum, synovial fluid and synovial tissue of patients developing rheumatoid arthritis during the first 12 weeks but at lower levels in long-term ones

useful to reflect local pathological changes

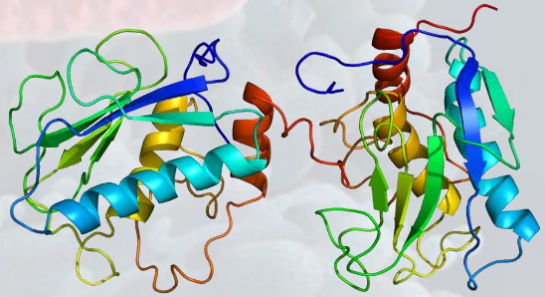


MATRIX METALLOPROTEINASE-3 (MMP-3)

synthesized and secreted by inflamed synovium and stimulated chondrocyte in response to cytokines in the joints

actively involved in joint destruction in rheumatoid arthritis patients

useful biomarker to evaluate the progression of the disease



MAGNETIC BEAD-BASED ELECTROCHEMICAL IMMUNOSENSORS

ELECTROCHEMICAL IMMUNOSENSORS

high sensitivity

high selectivity

quick and simple procedures

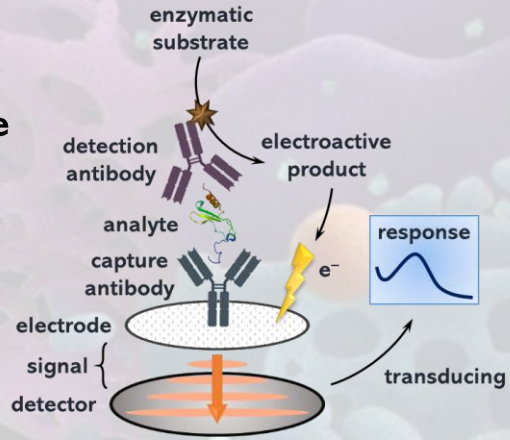
short response times

compatible with turbid samples

disposable

portable

inexpensive



MAGNETIC BEADS

high versatility

lower detection limits

minimal matrix effects

small volume of reagents, sample, and waste produced

SCREEN-PRINTED CARBON ELECTRODES

miniaturization

small sample volume

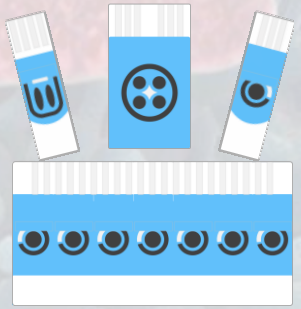
disposable

portability

mass production

versatility to customize

specific and multiplex applications



higher sample throughput

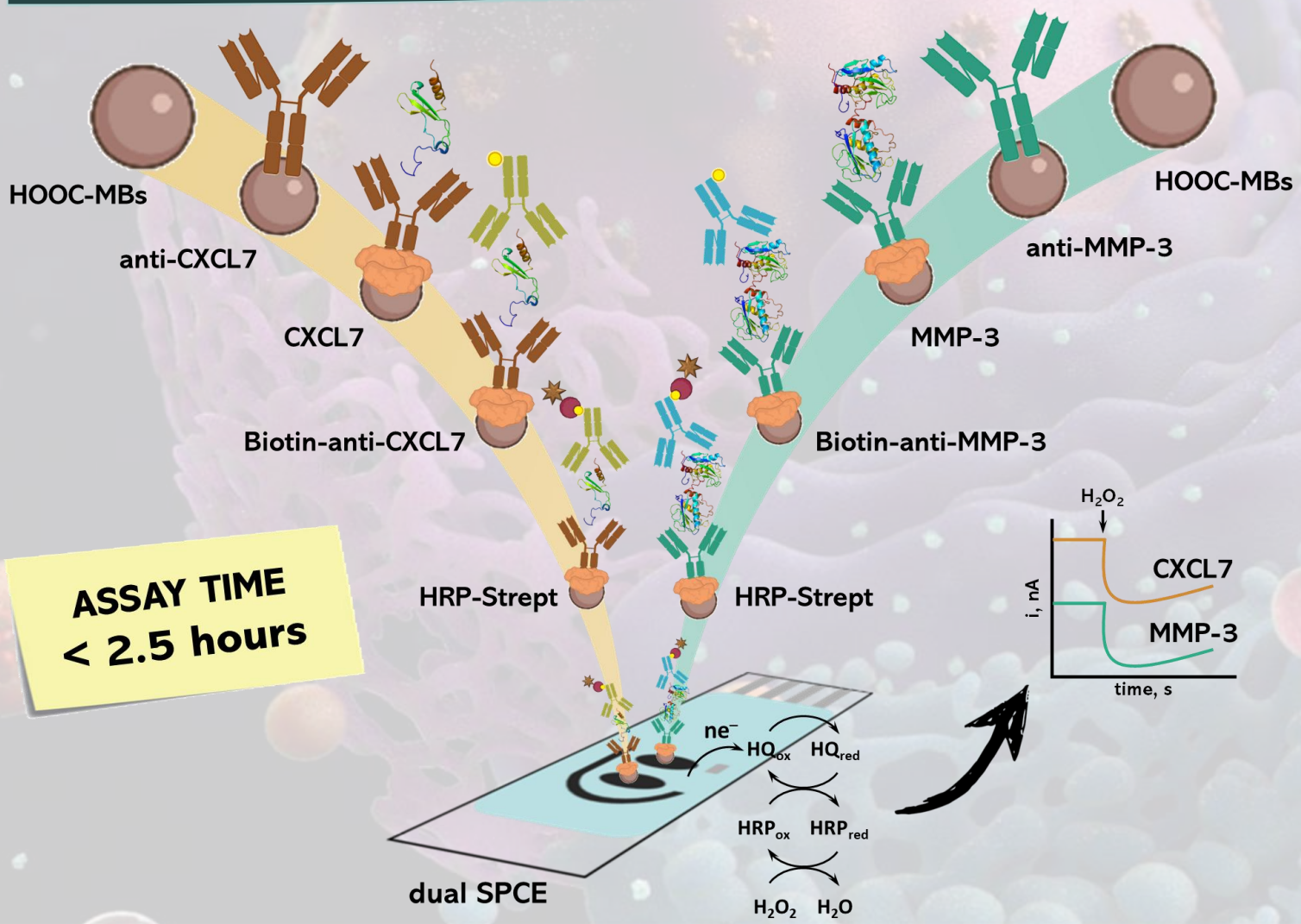
less sample consumption

reduced turnaround times

improved test efficiency

more reasonable cost

SIMULTANEOUS MATRIX METALLOPROTEINASE-3 AND NEUTROPHIL ACTIVATING PROTEIN-2 DETERMINATION



CXCL7

STUDIED VARIABLE	EVALUATED RANGE	SELECTED VALUE
[anti-CXCL7], $\mu g \cdot mL^{-1}$	2.5 – 25	10
$t_{anti\text{-}CXCL7}$, min	30 – 75	45
$t_{blocking}$, min	0 – 45	30
[Biotin-anti-CXCL7], $\mu g \cdot mL^{-1}$	0.05 – 0.5	0.25
$t_{Biotin\text{-}anti\text{-}CXCL7}$, min	30 – 90	60
[HRP-Strept], dil	1/4000 – 1/250	1/2000
$t_{HRP\text{-}Strept}$, min	10 – 30	20

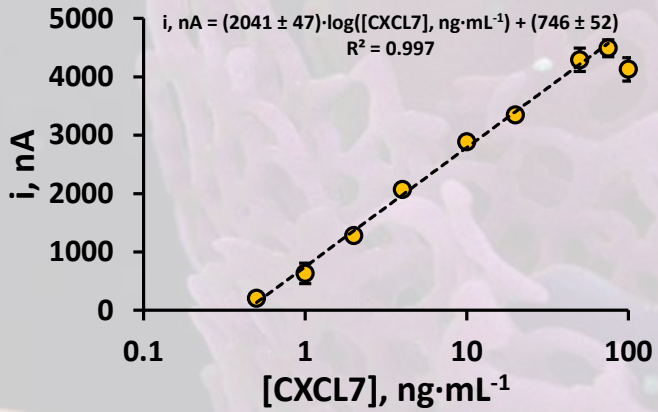
MMP-3

STUDIED VARIABLE	EVALUATED RANGE	SELECTED VALUE
[anti-MMP-3], $\mu g \cdot mL^{-1}$	5 – 20	15
$t_{anti\text{-}MMP\text{-}3}$, min	30 – 75	45
$t_{blocking}$, min	0 – 45	30
[Biotin-anti-MMP-3], $\mu g \cdot mL^{-1}$	0.5 – 2	1
$t_{Biotin\text{-}anti\text{-}MMP\text{-}3}$, min	15 – 60	45
[HRP-Strept], dil	1/1500 – 1/250	1/500
$t_{HRP\text{-}Strept}$, min	10 – 30	20

SIMULTANEOUS MATRIX METALLOPROTEINASE-3 AND NEUTROPHIL ACTIVATING PROTEIN-2 DETERMINATION

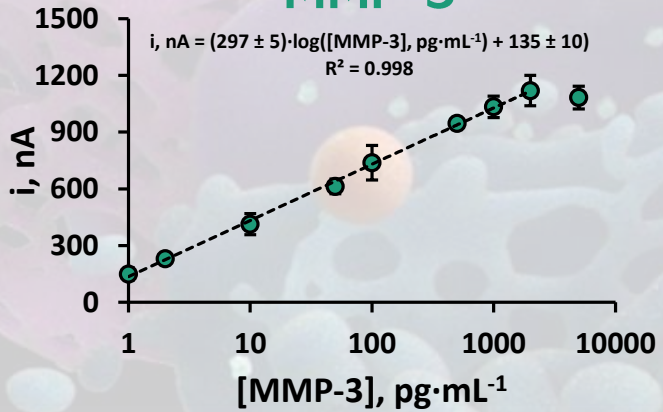
CALIBRATION PLOTS AND ANALYTICAL CHARACTERISTICS

CXCL7



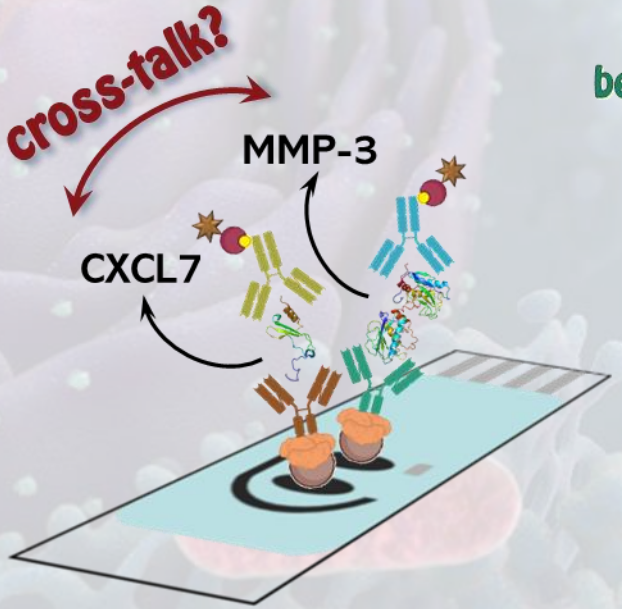
LINEAR RANGE:
1 – 75 ng·mL^{-1}
R^2 = 0.997
LOD: 0.8 ng·mL^{-1}

MMP-3

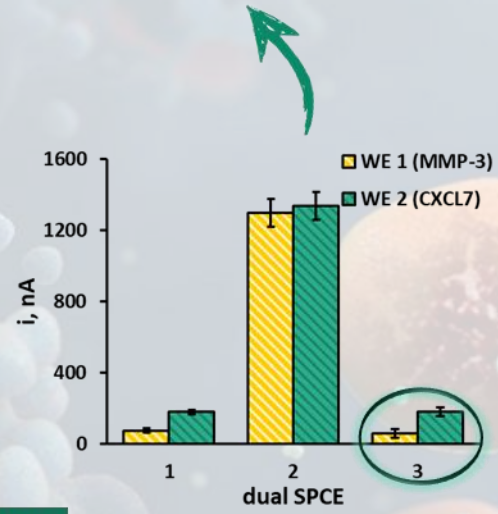


LINEAR RANGE:
2 – 2000 pg·mL^{-1}
R^2 = 0.998
LOD: 1.2 pg·mL^{-1}

EVALUATION OF CROSS-TALK



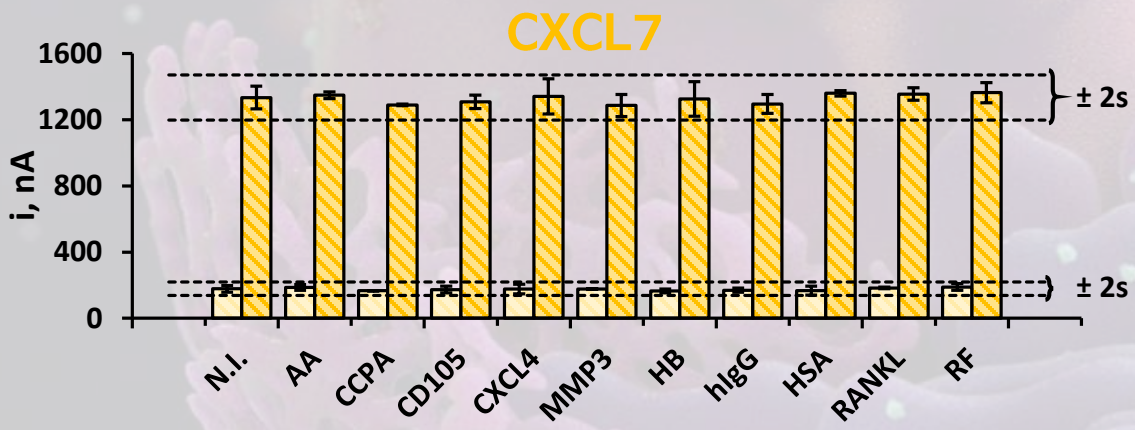
NO CROSS-TALK
between working electrodes!

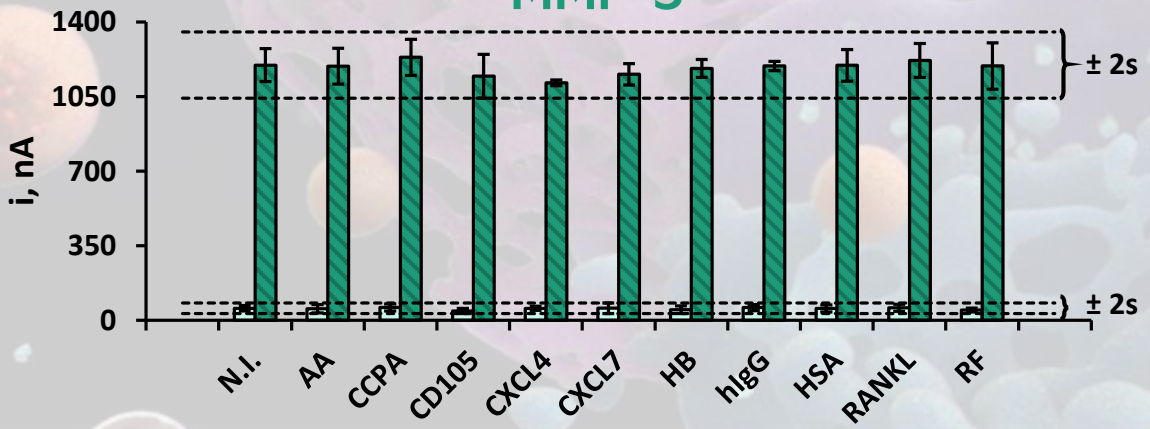


DUAL	WE 1 (anti-MMP-3)	WE 2 (anti-CXCL7)
1	[MMP-3] = 0 ng·mL^{-1}	[CXCL7] = 0 ng·mL^{-1}
2	[MMP-3] = 2 ng·mL^{-1}	[CXCL7] = 2 ng·mL^{-1}
3	[CXCL7] = 2 ng·mL^{-1}	[MMP-3] = 2 ng·mL^{-1}

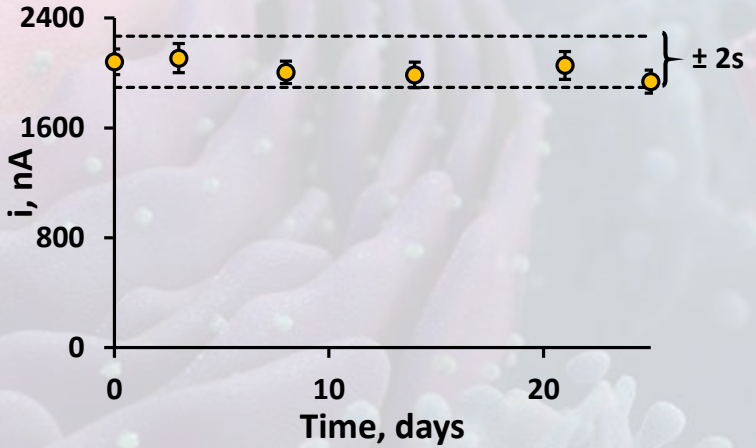
SIMULTANEOUS MATRIX METALLOPROTEINASE-3 AND NEUTROPHIL ACTIVATING PROTEIN-2 DETERMINATION

SELECTIVITY



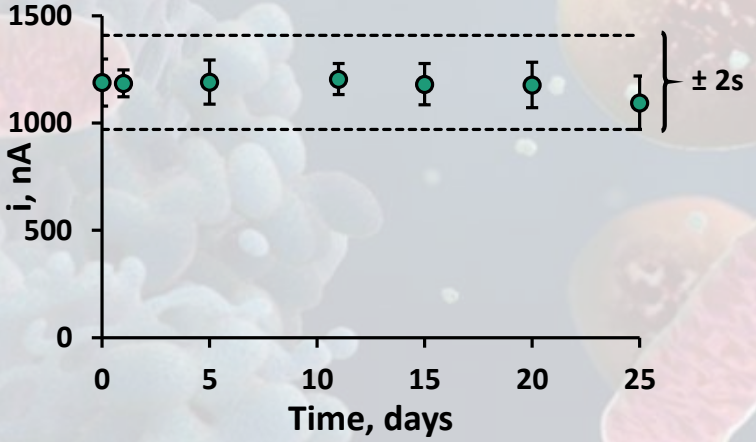


STABILITY



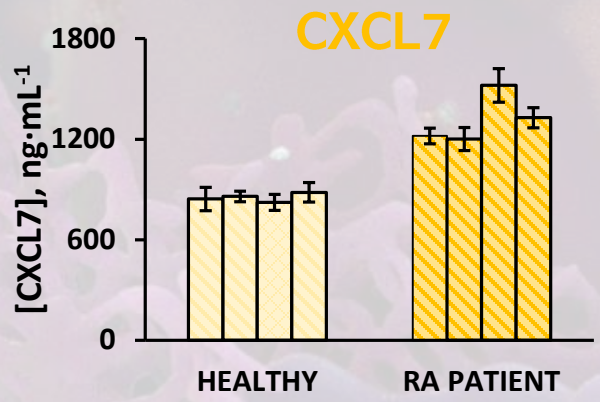
The immunoconjugate anti-CXCL7-HOOC-MBs can be stored in humidity at 8°C, being stable for at least 25 days

The immunoconjugate anti-MMP-3-HOOC-MBs can be stored in humidity at 8°C, being stable for at least 25 days



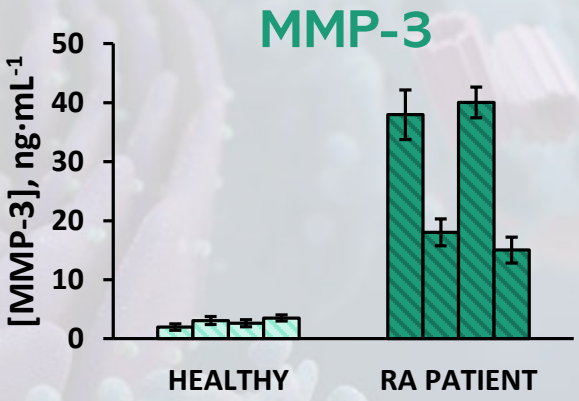
SIMULTANEOUS MATRIX METALLOPROTEINASE-3 AND NEUTROPHIL ACTIVATING PROTEIN-2 DETERMINATION

SERUM SAMPLE ANALYSIS



1/50 dilution in PBS pH 7.4

NO MATRIX EFFECT!!



SAMPLE	BIOSENSOR, ng·mL^{-1} (n = 4)	ELISA, ng·mL^{-1} (n = 2)
Healthy 1	843 ± 70	832 ± 81
Healthy 2	858 ± 32	860 ± 65
Healthy 3	823 ± 48	821 ± 73
Healthy 4	883 ± 57	891 ± 58
RA 1	1219 ± 47	1226 ± 51
RA 2	1521 ± 100	1513 ± 72
RA 3	1328 ± 60	1308 ± 65
RA 4	1201 ± 69	1195 ± 57

SAMPLE	BIOSENSOR, ng·mL^{-1} (n = 4)	ELISA, ng·mL^{-1} (n = 2)
Healthy 1	2.0 ± 0.5	2.0 ± 0.7
Healthy 2	3.1 ± 0.7	3.1 ± 0.8
Healthy 3	2.6 ± 0.6	3 ± 1
Healthy 4	3.5 ± 0.5	3.4 ± 0.8
RA 1	38 ± 4	39 ± 5
RA 2	40 ± 3	40 ± 6
RA 3	15 ± 2	17 ± 4
RA 4	18 ± 2	19 ± 3

CONCLUSIONS AND FUTURE WORK

1. The preparation and implementation of a dual immunosensor for the simultaneous determination of neutrophil activating protein-2 and matrix metalloproteinase-3 was described for the first time

2. The proposed method exhibits better analytical characteristics in terms of sensitivity, clinically relevant linear range, reproducibility, storage stability and selectivity than conventional ELISA immunoassays. In addition, the proposed immunosensor require shorter analysis times and less reagent consumption

3. The application of the immunosensor to the determination of these biomarkers in serum samples has provided good results

4. The obtained results show that the initial objective of developing sensitive, reliable and robust analytical devices for the determination of these biomarkers in complex clinical samples, and, therefore, suitable to develop point-of-care devices was reasonably achieved

ACKNOWLEDGEMENTS

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