Electronic Supplementary Material (ESI) for Materials Advances.

Gliadin-coated Gold Nanoparticles for Rapid Colorimetric Test for Celiac Disease

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ESI

S1 calculation of the molar extinction coefficient of Gliadin and BSA

The ProtParam tool (ExPasy Bioinformatics Resource Portal) was used to compute the molar extinction coefficient of gliadin and the peptide sequence. Gliadin was specified as a Swiss-Prot accession number (P02863). The computation was carried out on the complete sequence of gliadin composed of 286 amino acids. BSA is specified as a Swiss-Prot accession number (P02769). The extinction coefficient was calculated on the complete sequence composed of 607 amino acids. The results of the analysis are described in Table S1.

Table S1 Molecular extinction coefficient of Gliadin and BSA

Protein/Peptide	Molecular weight	Extinction coefficient
Gliadin	31 kDa	19285 $M^{-1}cm^{-1}$
BSA	66.5 kDa	47790 $M^{-1}cm^{-1}$

S2 Spectral data of Gliadin-coated AuNPs

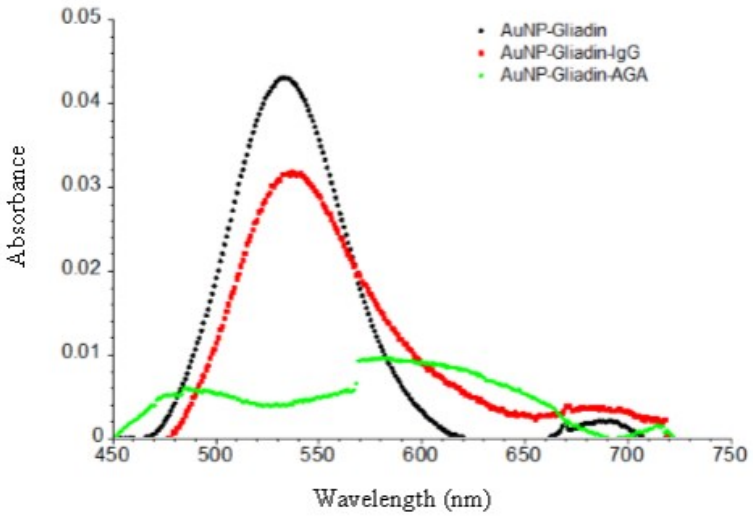

Figure S2*. Typical UV-Vis spectra of gliadin-coated AuNPs (black), upon incubation with control IgG (red) and with AGA (green).*

S3 Testing the effect of the addition of AGA at a level normally found in serum to uncoated 20 nm AuNPs.

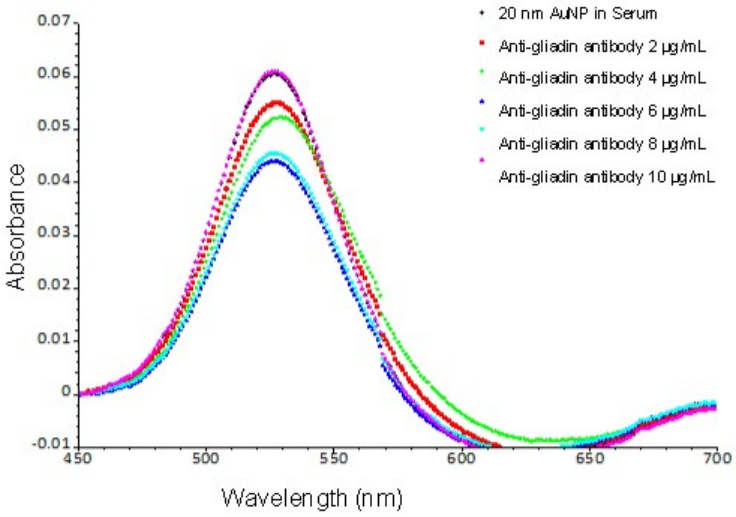

Figure S3. *Incubation of uncoated AuNPs in serum with AGA at various dilutions.*

S4 Testing the effect of the addition of AGA at a level normally found in serum to AuNPs coated with BSA.

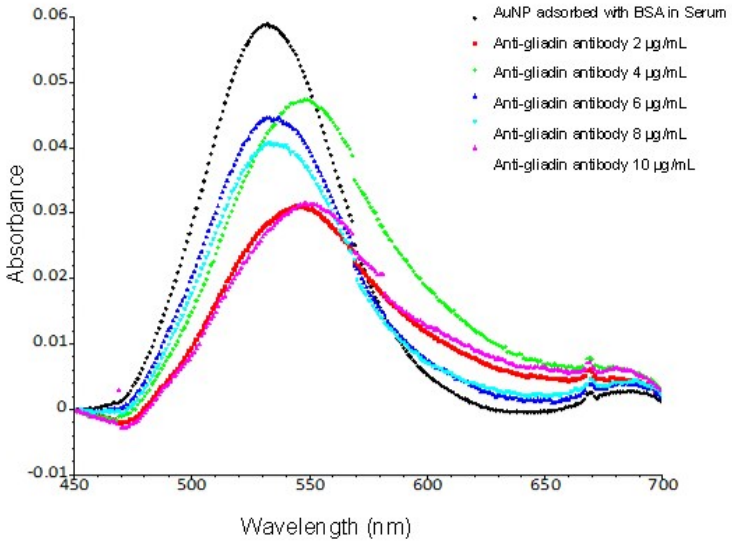

Figure S4. *Incubation of BSA coated AuNPs in serum with AGA at various dilutions.*

BSA has an isoelectric point (pI) of 4.6, therefor at neutral pH, it display a negative charge (zeta potential ~10 mV). Although an antibody (such as AGA) molecule is specific to the antigenic determinant to which it binds, the specificity is further related to the affinity and avidity of the antibody. Antibody affinity is associated with the preciseness of a stereochemical fit of an antibody combining sites to its complementary antigen determinant. It is, however, commonly observed that some antigenic determinants are shared between molecules, most likely due to electrostatic and hydrogen interaction. In such a scenario, some antibodies that are induced in response to one antigen may combine with another antigen as well by cross-reacting leading to a stronger effect on BSA coated AuNPs comparing with uncoated AuNPs.

S5 Statistical analysis of values for samples with AuNP adsorbed with gliadin in the presence of AGA antibody and the control antibody (IgG from rabbit serum) at dilutions 2-10 µg/mL.

Table (S5) Shows the calculated p value in AuNP adsorbed with gliadin in the presence of AGA antibody and the control antibody (IgG from rabbit serum) at dilutions 1-10 µg/mL.

Sample	Percentage Absorbance	*t-test* p -values
AuNP adsorbed with gliadin	100	
Anti-gliadin antibody 2.0 µg/mL	54	0.004
Control antibody 2.0 µg/mL	83	
Anti-gliadin antibody 4.0 µg/mL	45	0.002
Control antibody 4.0 µg/mL	80	
Anti-gliadin antibody 6.0 µg/mL	36	0.001
Control antibody 6.0 µg/mL	80	
Anti-gliadin antibody 8.0 µg/mL	33	0.001
Control antibody 8.0 µg/mL	72	
Anti-gliadin antibody 10.0 µg/mL	40	0.004
Control antibody 10.0 µg/mL	68	

The assay sensitivity in spiked serum and saliva was calculated as Colorimetric Response = I max at 580 nm/ I at 532 nm i.e. absorbance value obtained at 580 nm. This is the wavelength

where a shift in absorbance is observed following the interaction of the antibody with the AuNP coated with gliadin in serum/saliva divided by the maximum absorbance value of AuNP coated with gliadin in serum/saliva.

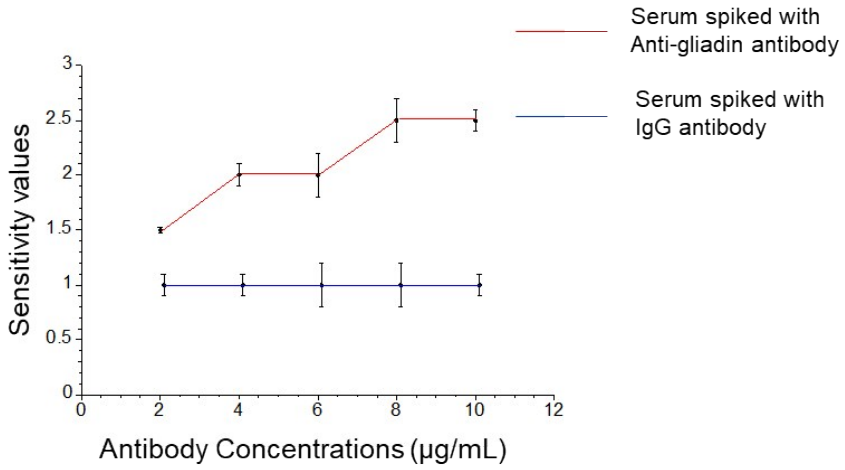

Figure S6. Colorimetric response curve plotted in AuNP adsorbed with gliadin in 1:10 diluted serum following the addition of AGA antibody at dilutions 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL.

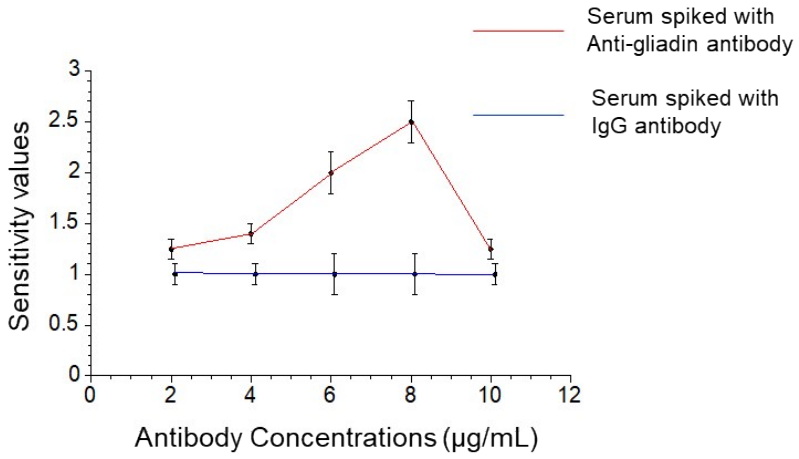

Figure S7. Colorimetric response curve plotted in AuNP adsorbed with gliadin in 1:50 diluted serum following the addition of AGA at dilutions 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL.

Table S8. Comparison of the patient samples analyzed using the AuNP-AGA test with the previously existing histology and serological* results

Volunteer	Histology	tTG-IgA	DGP-IgG	AuNP-AGA Test
n.1	CD	1 (<4)	3 (<20)	CD Positive
n.2	Non-CD	0.1 (0-6)	0.2 (0-6)	CD Negative
n.3	CD	>100 (<4)	33 (<20)	CD Positive
n.4	CD	121 (<20)		CD Positive
n.5	CD	>100 (<4)	>100 (<20)	CD Positive
n.6	CD	217 (<5)	>150 (<20)	CD Positive
n.7	CD	13 (0-6)	23 (0-6)	CD Positive
n.8	CD	>100 (<5)	>100 (<20)	CD Positive
n.9	CD	11 (0-6)	1.4 (0-6)	CD Positive
n.10	CD	9 (<4)	97 (<20)	CD Positive
n.11ǂ	CD	18.2 (0<20)	3 (0.20)	CD Negative
n.12ǂǂ	CD	16 (0-20)	7 (<20)	CD Positive
n.13	Non-CD	4 (0-20)		CD Negative
n.14	CD	47 (<5)	86 (<5)	CD Positive
n.15	CD	74 (0-20)		CD Positive
n.16	CD	145 (0-20)		CD Positive
n.17	CD	57 (<4)	93 (<20)	CD Positive
n.18	CD	149 (<20)	63 (<20)	CD Positive
n.19	CD	>100 (<4)	>100 (<20)	CD Positive
n.20ǂǂǂ	Non-CD	3.8 (<6)	37 (<6)	CD Positive
n.21	Non-CD	<5 (<5)	<20 (<20)	CD Negative
n.22	CD	180 (0-6)	21 (0-6)	CD Positive
n.23	CD	20 (0-6)	8.1 (0-6)	CD Positive

* Serology tTG (tissue transglutaminase), DGP (Deaminated gliadin peptides) results are indicated as IgA or IgG levels followed by normal reference ranges in brackets.

ǂ Following GFD > 8 weeks, ǂǂ Following GFD < 2 weeks, ǂǂǂ False-positive based on histology and tTG antibody titre.

Table S9. Analysis of 7 samples with potential or latent CD using the AuNP-AGA test as compared with previously existing serology*. Cases are separated into those with

histological observations of patchy /irregular Mucosal lesions or those with mucosal inflammation resulting from an increase in $\gamma\delta^+$ IELs.

Volunteer	Histology	tTG-IgA	DGP-IgG	AuNP-AGA Test
n.24	Mucosal lesions	5 (<20)	17 (<20)	CD Positive
n.25	Mucosal lesions	28 (0-6)	22 (0-6)	CD Positive
n.26	Mucosal lesions	4.8 (0-6)	22 (0-6)	CD Positive
n.27	Increased $\gamma\delta^+$ IELs	12 (0-6)	18 (0-6)	CD Positive
n.28	Increased $\gamma\delta^+$ IELs	11 (0-6)	13 (0-6)	CD Positive
n.29	Increased $\gamma\delta^+$ IELs	<5 (<5)	<20 (<20)	CD Positive
n.30	Increased $\gamma\delta^+$ IELs	<5 (<5)	22 (<20)	CD Positive

* Serology tTG (tissue transglutaminase), DGP (Deaminated gliadin peptides) results are indicated as IgA or IgG levels followed by normal reference ranges in brackets, IELs (Intraepithelial lymphocytes).