Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Dual enzyme-inorganic hybrid nanoflowers incorporated microfluidic paper-based analytic devices (μPADs) biosensor for sensitive visualized detection of glucose

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Fig. S1. SEM images of GOx nanoflowers prepared in the presence of $CuSO_4$ in PBS (pH 7.4) at different enzyme concentrations of 0.01 $mg \cdot mL^{-1}$ (A), 0.05 $mg \cdot mL^{-1}$ (B), 0.1 $mg \cdot mL^{-1}$ (C), and 0.5 $mg \cdot mL^{-1}$ (D). The diameter for nanoflowers obtained in different cases is 28± 3 μm, 20± 5 μm, 19± 2 μm and 15± 1 μm, respectively.

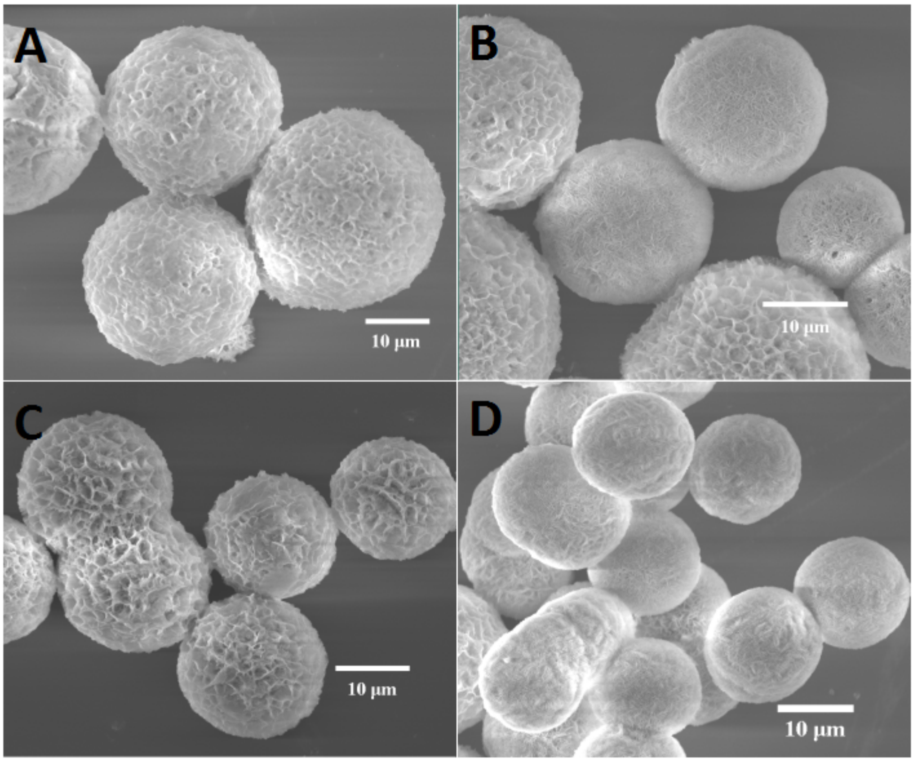

Fig. S2. SEM images of GOx&HRP-$Cu_3(PO_4)_2$ nanoflowers prepared in PBS solution (pH 7.4) containing GOx concentration of 0.5 mg·mL^{-1} and different concentration of HRP: (A) 0.01 mg·mL^{-1}, (B) 0.1mg·mL^{-1}, (C) 0.5 mg·mL^{-1}. The diameter for nanoflowers obtained in different cases is 20± 2 μm, 20± 1 μm, 19± 2 μm, respectively.

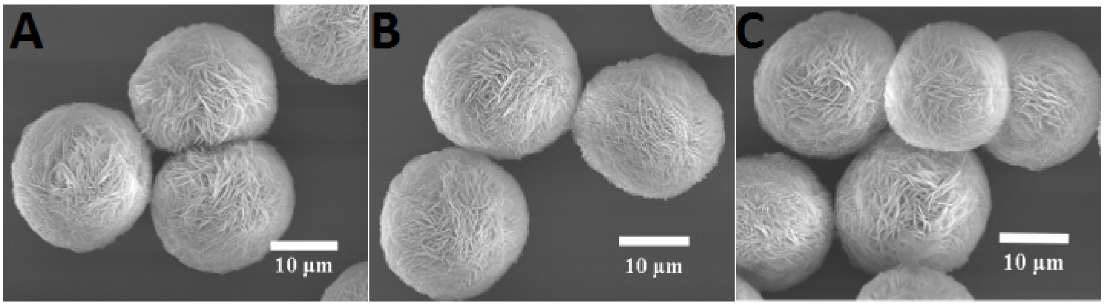

Fig. S3. SEM images for formation of GOx&HRP-$Cu_3(PO_4)_2$ nanoflowers at 2 h (A), 12 h (B), 36 h (C), 72 h (D). The diameter for nanoflowers obtained in different cases is 1.8± 0.4 μm, 15± 2 μm, 14± 2 μm and 16± 2 μm, respectively.

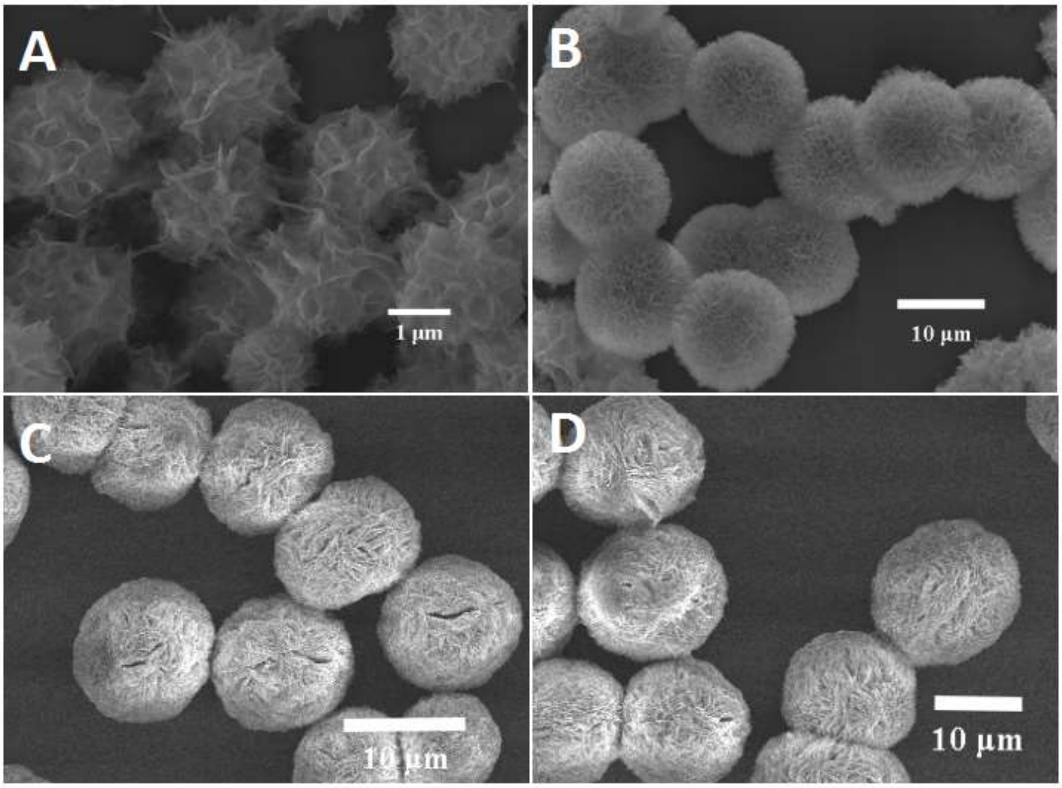

Fig. S4. XRD patterns of particles: (A) nanoflowers acquired with GOx and HRP; (B) particles of crystals acquired without enzymes; (C) standard $Cu_3(PO_4)_2$ (JPSCD00-022-0548)

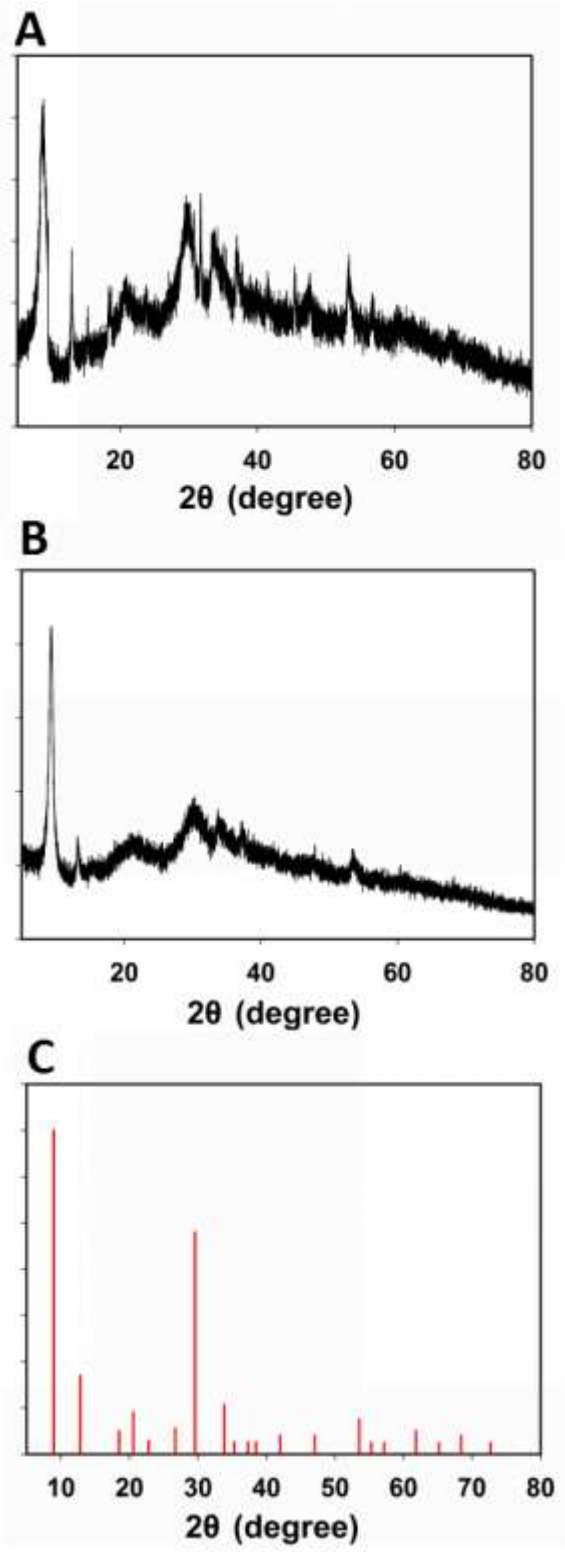

Fig. S5 EDX spectrum of the GOx&HRP-$Cu_3(PO_4)_2$ nanoflowers.

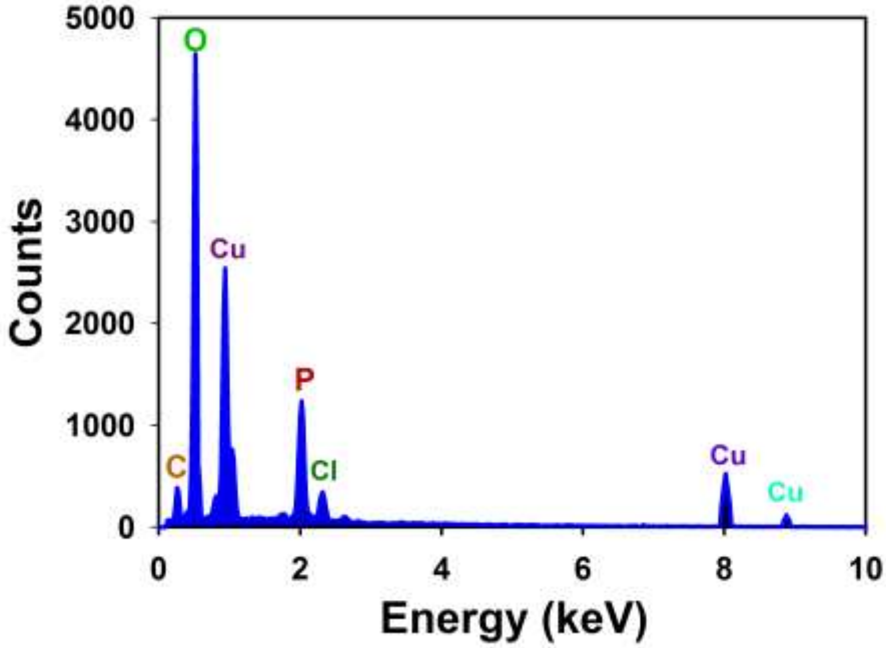

Fig. S6. Photograph of μPADs used for detection of 2 mM glucose with GOx&HRP-$Cu_3(PO_4)_2$ nanoflowers prepared using different reaction times. (A) 12 h, (B) 36 h.

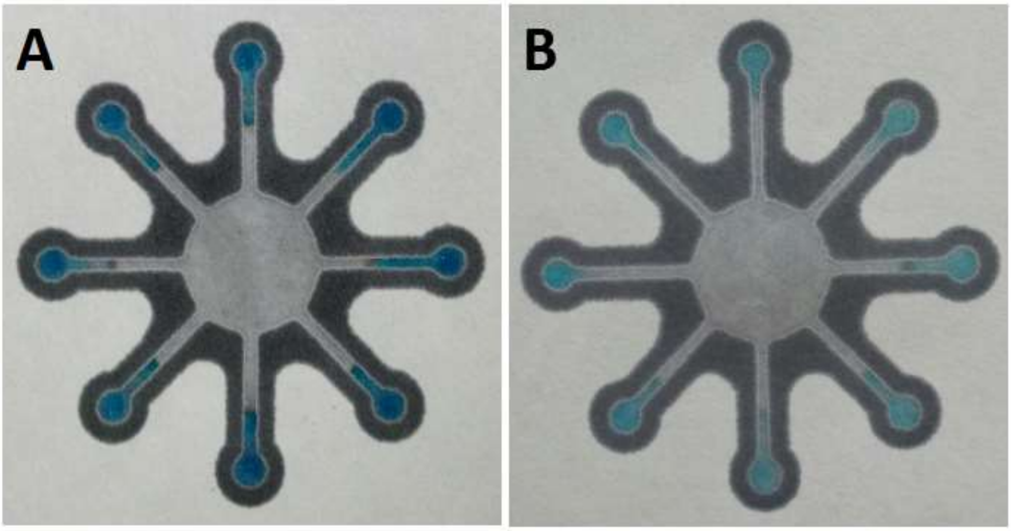

Fig. S7. Photograph of µPADs (A) and the grayscale image (B). The grayscale values were used to analyze the color intensity and gradient by ImageJ software.

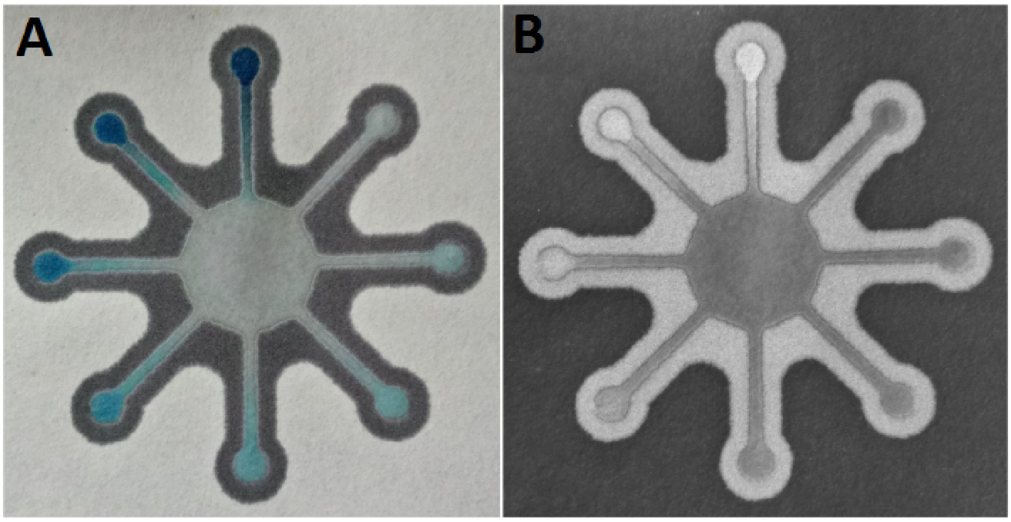

Fig. S8. Photograph of μPADs used for the detection of glucose in the whole blood samples. The glucose levels from A to H are 2.8, 3.4, 4.2, 4.6, 5.7, 7.9, 8.9 and 7.2 mM (The original concentrations of G and H are 11.9 and 14.4 mM repectively).

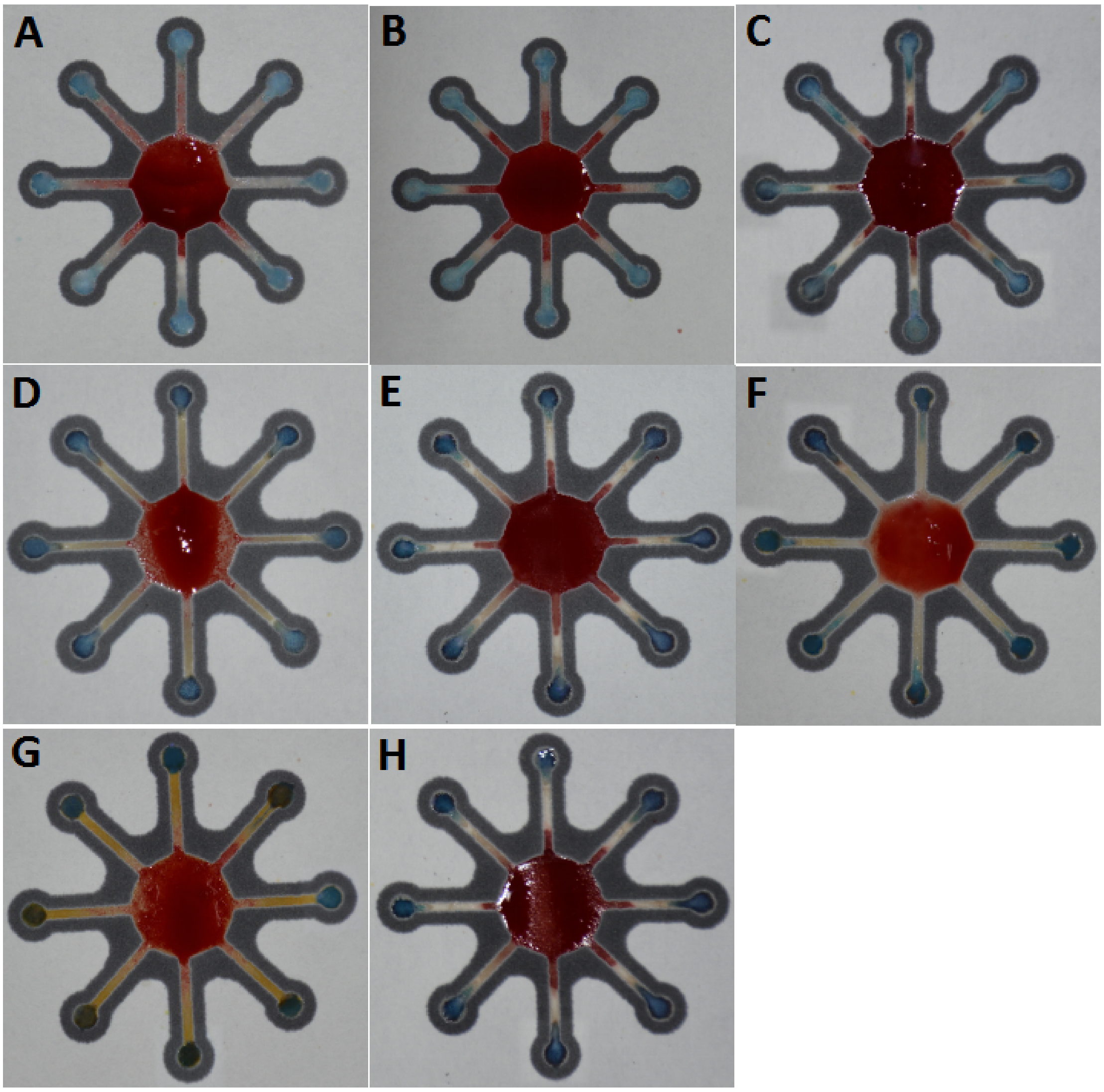

Table S1 Comparison of the developed assay with literature reports for glucose detection.

Materials used	Device	Analytical range (mM)	Limit of detection (mM)	Reference
GOx	μPED	5.8-31.3	0.2	[1]
GOx	μPED	0.2-22.2	0.22	[2]
GOx	μPED	2.9-33.1	----	[3]
GOx	μPED	1-5	----	[4]
GOx	μPED	2-100	0.21	[5]
AuNPs, TMB	spectrophotometer	0.018-1.1	0.004	[6]
GOx, Fe_3O_4 MNPs	spectrophotometer	0.05-0.1	0.03	[7]
GOx, M4NRASP	μPAD	0.42-50	0.14	[8]
GOx, HRP and KI	μPAD	2-12	0.7	[9]
GOx, HRP, 4-AAP and TOPs	μPAD	0.05-1 0.3-1	0.038 0.21	[10]
GOx, HRP, 4-APP and TBHBA	μPAD	1-11	0.3	[11]
GOx and 1,10-phenanthroline	μPAD	0.5-5	----	[12]
GOx, HRP and KI	μPAD	2.5-50	----	[13]
GOx ,HRP, 4-AAP and MAOS	μPAD	5-17	0.3	[14]
GOx-HRP nanoflower, TMB	μPAD	0.1-10	0.025	Our work

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