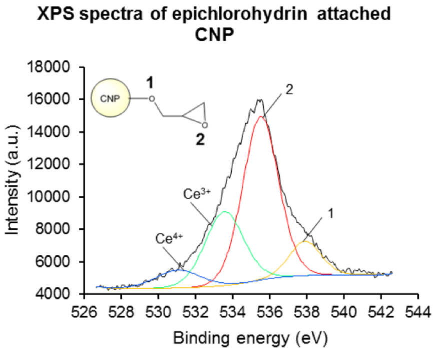
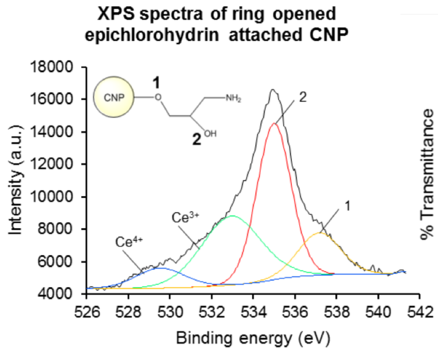
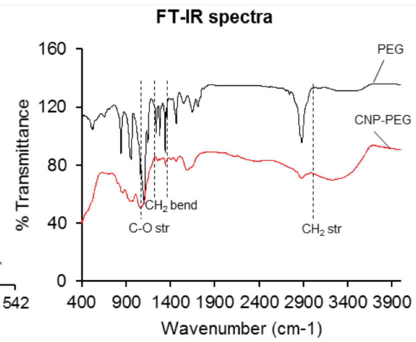
Q1. Are the cerium oxide nanoparticles are chemically bonded to the GOX via the PEG spacer?

Answer) Yes. Cerium oxide nanoparticles (CNP) and glucose oxidase (GOX) were chemically linked by PEG (polyethylene glycol) spacer. To synthesize CNP-PEG-GOX, we introduced functional amine groups on the surface of CNP (CNP-NH2), 2) PEGylated amine-modified CNP (CNP-PEG) and 3) conjugated CNP-PEG with GOX.

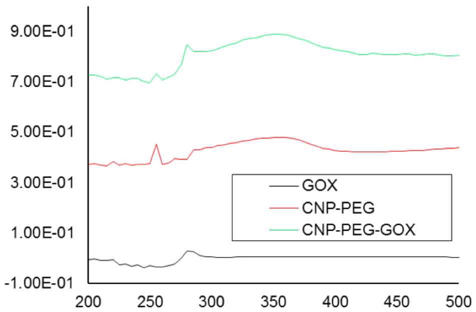
Results



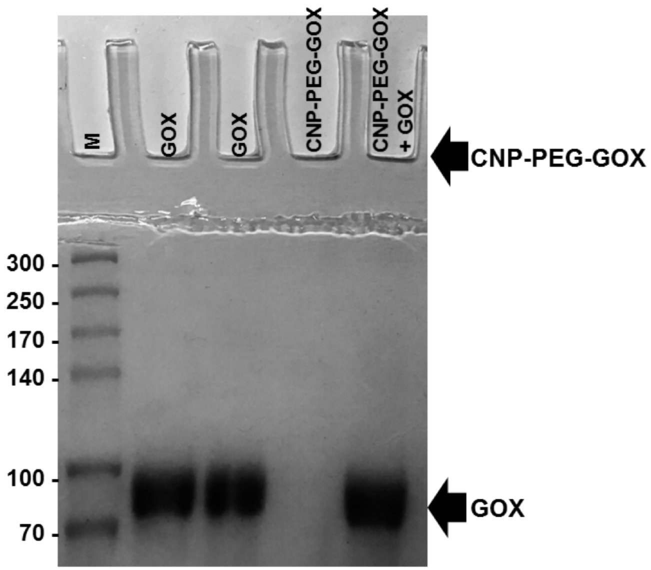




This data shows shifting of the epichlorohydrin O peak from 535.52 to 534.99 eV. This result demonstrates that amine groups formed on the surfaces of the CNPs in the process of epoxide ring opening. The PEGylation of CNP-NH_2 was confirmed by FT-IR spectroscopy. Reaction results of NHS-PEG-COOH with CNP-NH_2, CH_2 str (2900 cm^{-1}), CH_2 bend (1300-1400 cm^{-1}) and C-O str peak (1100 cm^{-1}) are shown in the data. These peaks are attributed to binding amine-modified-CNPs with PEG.



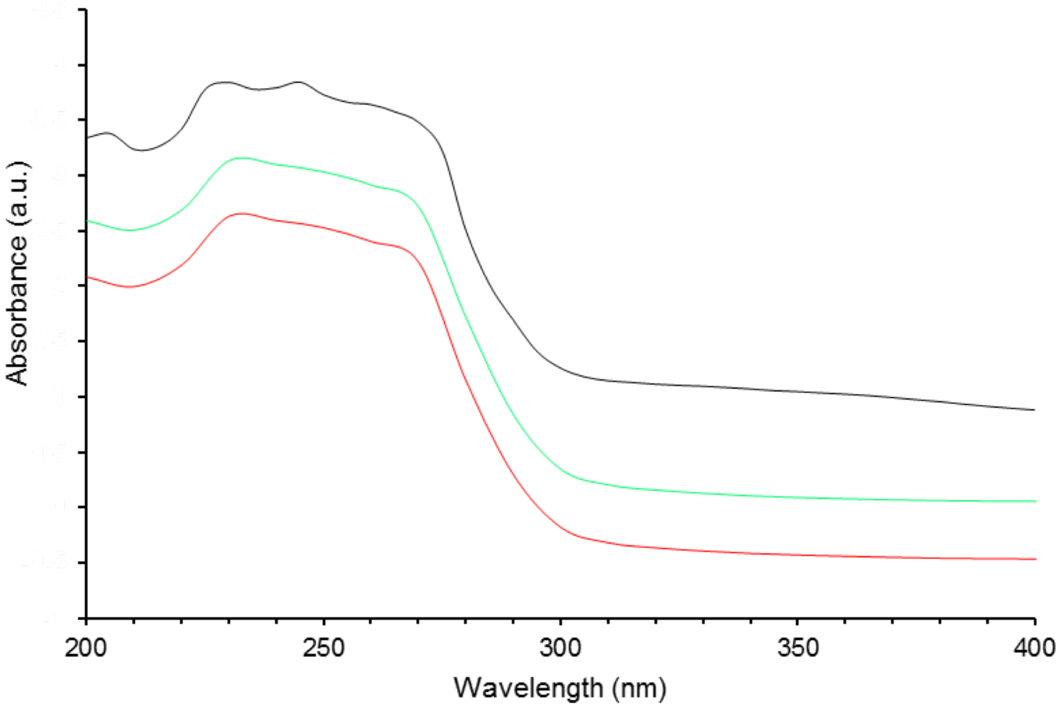
The synthesis of CNP-PEG-GOX was confirmed by peak shifting in UV-vis absorbance spectra. The broad peak around 255 nm of CNP-PEG disappeared and shifted to 280 nm in CNP-PEG-GOX as a result of conjugation with GOX.



We also evaluated the purity of CNP-PEG-GOX by comparing results of CNP-PEG-GOX + GOX, CNP-PEG-GOX and GOX in SDS-PAGE. Experimental result shows that the movement of sample in lane 3 is not seen due to aggregation of the CNP-PEG-GOX complexes. In addition, the specific GOX band is not seen in lane 3. In other words, CNP-PEG-GOX showed no residual GOX that did not react. This indicates that the CNP-PEG-GOX complexes have high purity.

Q2. Would there be any risks of the complex degrading after prolonged durations of contact with biofluids in the eye? Are there also any risks of the nanoparticles crossing the blood-brain barrier?

Answer) our contact lens has not yet tested on 'real' eye of patients because we need to approve clinical trials from Korea-FDA. But, when the nanoparticle-laden contact lens was stored in a solution for 1 month, we did not see any leaky nanoparticles in the solution. Also the morphology of nanoparticle-laden contact lens were still very good. The reason is that the nanoparticles are covalently linked with CNP-PEG-GOX. When the nanoparticles without chemical crosslinking were laden in contact lens, they leak out.

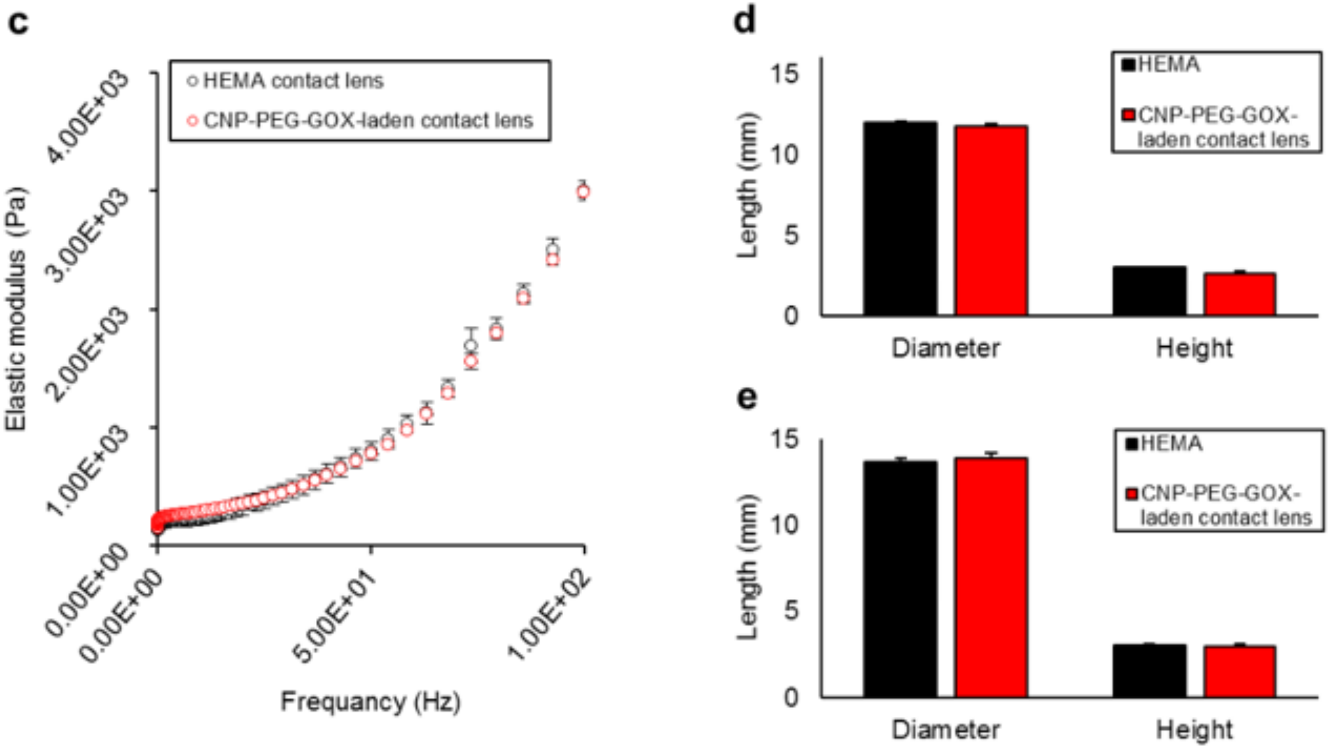


Absorbance spectra of CNPs and buffer solution before and after a month of storing the sensor. Absorbance peaks at 230 and 245 nm that were shown in CNP solution were not detected in stored buffer after a month of storing. It suggested that none of CNPs was detected in stored buffer. Black line = CNP; green line = buffer after a month of storing; red line = buffer before storing.

The CNP-PEG-GOX-laden contact lens was stored in 0.9% NaCl solution for 1 month. The structure of the contact lens was observed and the CNPs of the stored solution was confirmed by UV-Vis spectrum analysis (Supplementary Fig. 7). After 1 month, its original structure was maintained, and no 245 nm peak of cerium oxide was observed in the storage buffer. It means that CNP-PEG-GOX-laden contact lens has excellent stability. This result shows that CNP-PEG-GOX is stable even if there is no chemical bond between the HEMA polymer and CNP-PEG-GOX. This is because CNP-PEG-GOX is larger than the pore size of HEMA contact lenses. There is no need to consider the problem of reducing the reactivity of CNP-PEG-GOX because no chemical bond is required.

Q3. Is the contact lens sensor meant to be worn like normal contact lens?

Answer) Yes. To confirm that, we tested the elastic modulus values, hydration property, water contents in both normal conventional contact-lens and our nanoparticle-laden contact-lens. All parameter values in both lens are exactly same, which means that our nanoparticle-laden contact-lens could be wearable like conventional normal lens. In addition, we can easily fabricate our nanoparticle-laden contact lens using the conventional 'mold'.

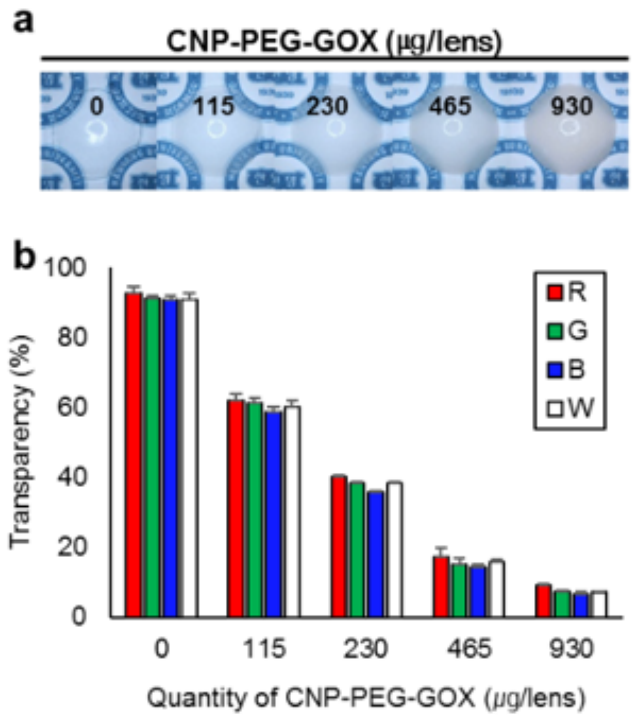


Elastic modulus of CNP-PEG-GOX-laden contact lens (930 μg/lens) and HEMA contact lens.

Change of diameter and height in HEMA contact lens and CNP-PEG-GOX-laden contact lens before hydration and after hydration.
Black bar = HEMA contact lens; red bar = CNP-PEG-GOX-laden contact lens.

Q4. After reaction, the contact lens turned deep yellow. Is the opacity of the contact lens changed also (I see that the rabbit's eyes in one of the images turned opaque)? Would the patient's vision be affected following the change in colour?

Answer) As you mentioned, lens itself became opaque after color development. So we re-designed a contact lens with transparent center as you can see the photo.

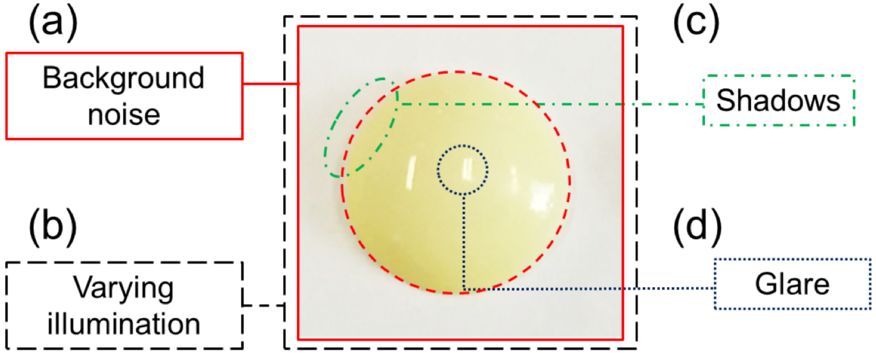


As shown in this data, the more CNP-PEG-GOX contained in the lens, the opaquer it becomes, and as the light transmittance shows transparency gradually decreases.

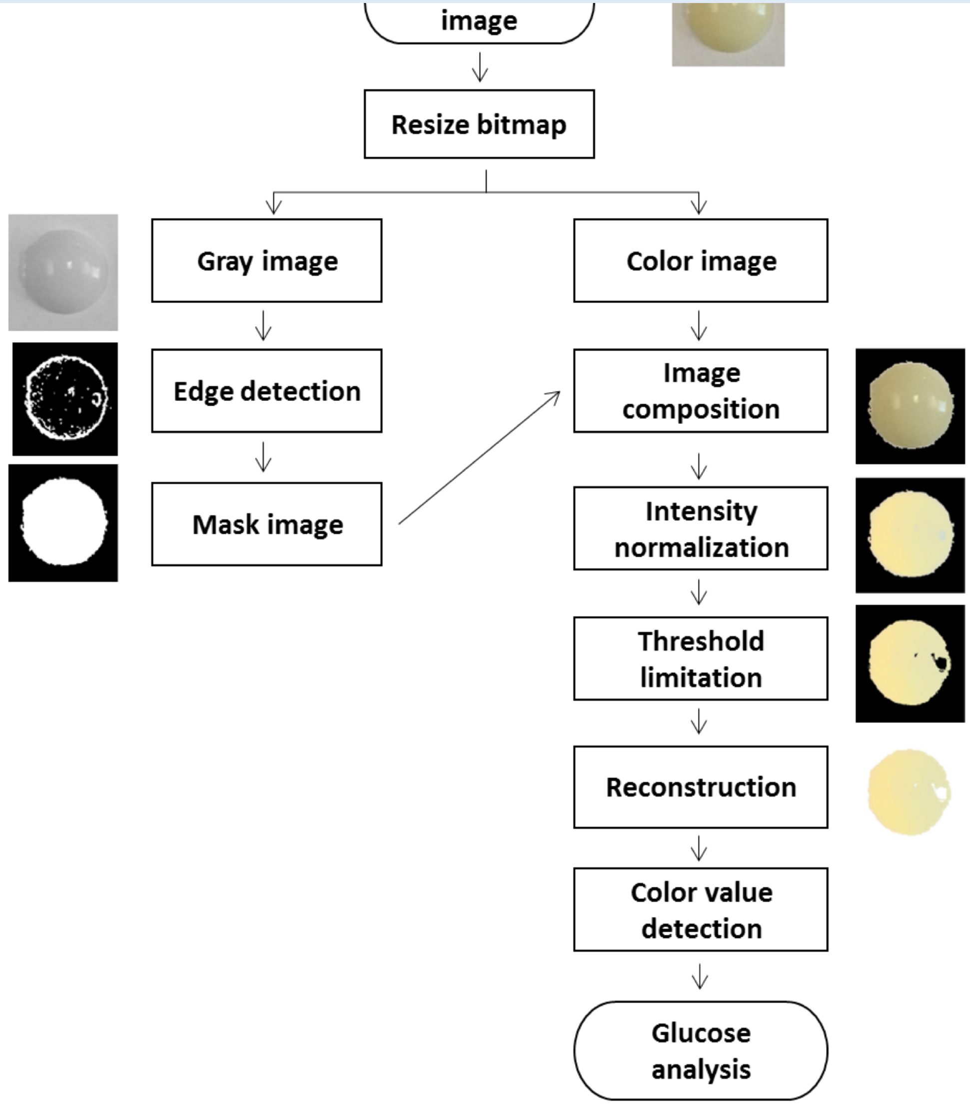
The CNP-PEG-GOX complex is opaque because it is mounted on the contact lens and causes a disadvantage in that the field of view is not secured. To solve this problem, a CNP-PEG-GOX-laden contact lens with a clear center was prepared.

Q5. Does the app capturing the image of the contact lens account for the differences in environmental conditions and lighting when the images are taken? Does it give a quantitative measurement of the glucose concentration?

Answer) We made an correction algorithm to take a exact color image from several environmental condition such as background noise, shadow of lens itself, glare and other illumination. And then we take a 'reconstructed' image that was quantified from the standard algorithm with known concentration of glucose.



Versatile noises created by inhomogeneous illumination; (a) external noise, (b) varying illumination, (c) shadow, and (d) glare.



Flow charts of the glucose colorimetry detection algorithms