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Flexible urea sensor fabrication by localized laser - induced pyrolysis of Kapton

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Introduction

We present a fabrication method of enzymatic urea sensor employing new, low-cost, lithography free, one-step process of laser-induced carbonization [1-4] and compare it to glassy carbon [5].

Here, we report fabrication of two types of enzymatic urea sensors by immobilization of urease:

- directly on carbon;
- via intermediate biopolymer (chitosan) layer.

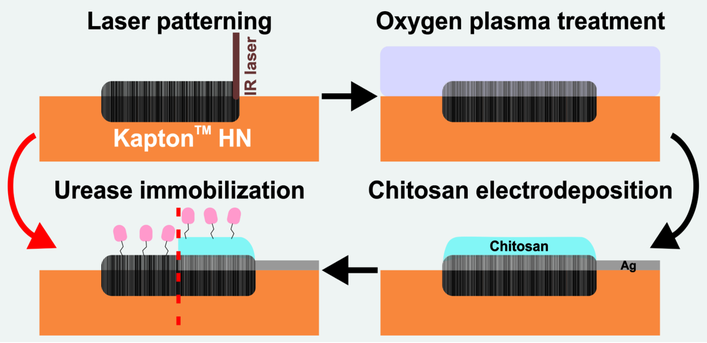

Fig. 1. Flow chart of the process

Carbon electrode fabrication and characterization

Fabrication:

- Kapton™HN 125±13µm;
- CO_2 laser, wavelength 10.6µm;
- Power 4.8 W, speed 10 cm/s.

Properties:

- High porosity:
  - BET surface area – 255 m^2/g;
  - Surface porosity – 15.2%;
- Composition:
  - XPS: C – 76.1%, N – 1.0%, O – 23.0%;
  - Combustion analyzer: C – 93%, N–0.9%, H – 0.4%;
- Conductivity – 7.1±0.7 S/cm;
- *d*-spacing – 3.48Å; L_a = 4.0nm; L_c = 7.1nm;
- Raman ratio (I_D/I_G) – 0.8;
- Hydrophilicity (contact angle 70°).

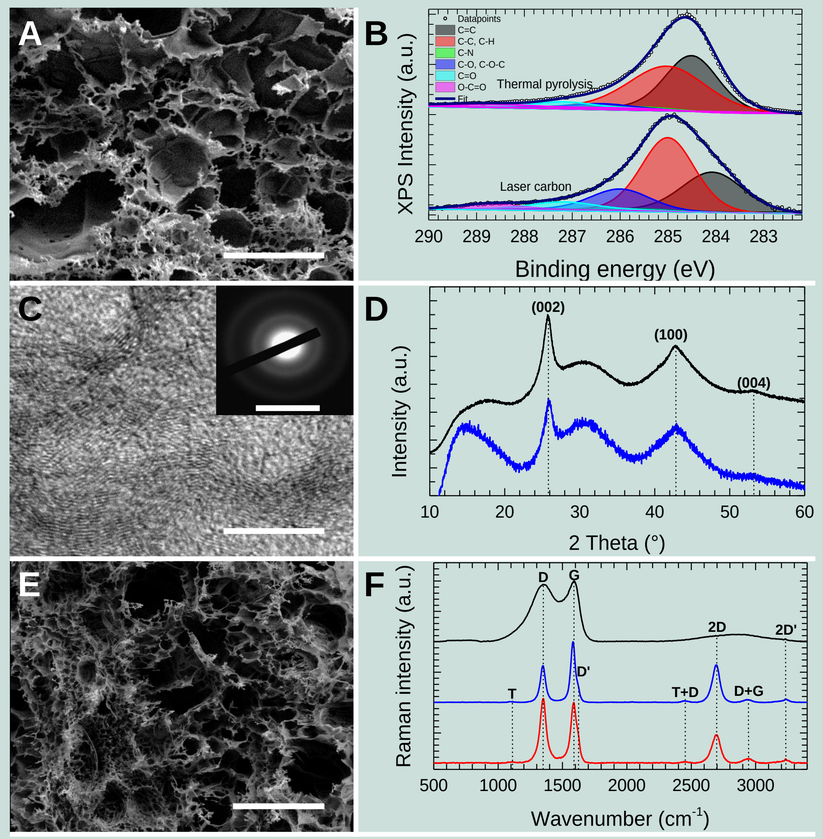

Fig. 2. (**A**) SEM image of fabricated laser carbon ([scale bar] =10µm); (**B**) XPS spectra C 1s line; (**C**) TEM image of laser carbon and corresponding selected area diffraction, ([scale bar] = 10 nm, [SAED]=10 1/nm) (**D**) powder X-ray diffractrogram collected from powder; (E) morphology after oxygen plasma treatment ([scale bar] = 10 µm); (F) Raman spectra. In all cases in black thermally pyrolysed Kapton at 900°C, blue - laser carbon, red – laser carbon after oxygen plasma treatment

Chitosan electrodeposition and process optimization

- Chitosan preparation: chitosan solution 1 % w/v, pH 5.5 in diluted HCl (0.2 M) and filtered,
- Improvement of hydrophilicity
  - Oxygen plasma treatment at 100 W for 120s
  - Contact angle <10°;
  - Retention over 5 days;
- Highest deposition quantity at current direction perpendicular to laser patterning;
- Optimal deposition duration is 15 min at 4 A/m^2. Increase of the time leads to chitosan self-peeling;
- Chitosan film thickness ~2 µm after drying.

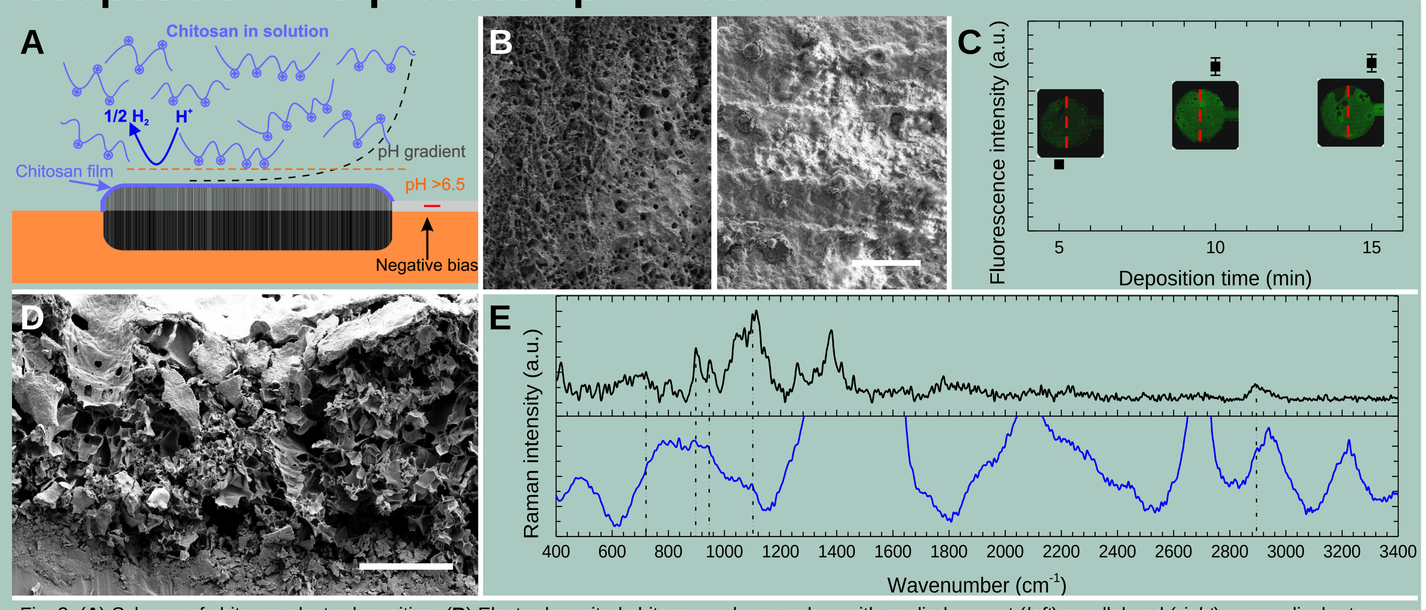

Fig. 3. (**A**) Scheme of chitosan electrodeposition. (**B**) Electrodeposited chitosan on laser carbon with applied current (*left*) parallel and (*right*) perpendicular to laser patterning direction ([scale bar] = 50 µm). (**C**) Quantity of chitosan deposition over time of attached chitosan determined with fluorescence microscopy. (**D**) Cross-sectional SEM. (**E**) Raman spectra collected from chitosan film deposited on gold (*black*) and on carbon electrode (*blue*)

Urea sensor fabrication and analysis

- Urease from Jack Beans (Canavalia ensiformis):
  - Covalently coupled to chitosan with glutaraldehyde (Sigma Aldrich 25% in water) [6];
  - All sensors were immobilized in 1 mg/ml urease solution overnight;
- Activity tested by urea hydrolysis and ammonia release [7,8].

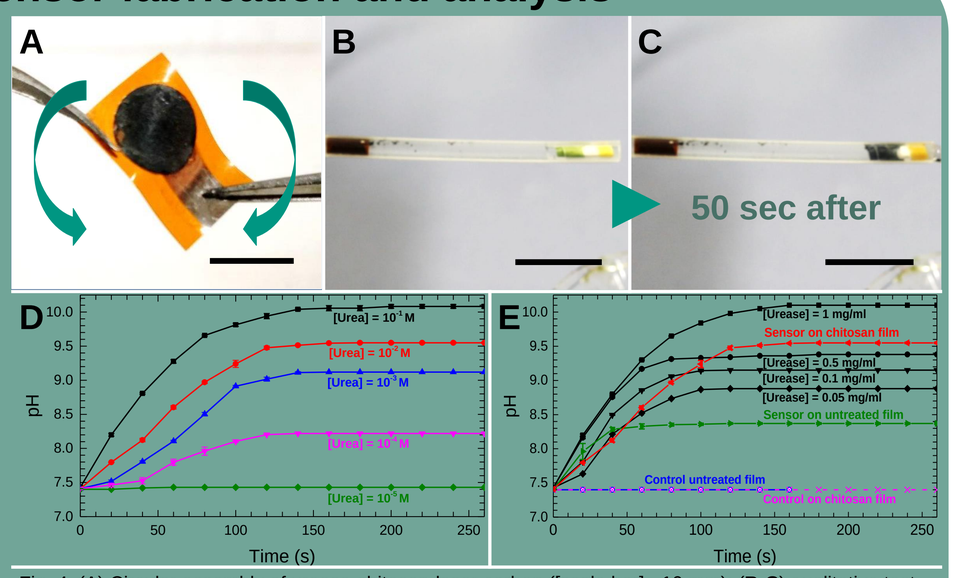

Fig. 4. (A) Circular assembly of urease-chitosan-laser carbon ([scale bar] =10 mm); (**B**-**C**) qualitative test with pH indicator ([scale bar] = 20 mm). (**D**) Urease activity at different urea concentrations, each sensor was coated with 1 mg/ml of initial urease concentration. (**E**) Comparison of urease activity to control solutions of various urea concentrations (*black*) and test sensors: urease-chitosan-laser carbon (*red*) and urease - laser carbon (*green*)

Conclusions

Material exhibits electrical, mechanical and physical properties different from glassy carbon.

Simplicity, flexibility and versatility of such method enable:

- fabrication of the patterns of various designs;
- variation of the properties dependent on applied laser parameters;
- electrodeposition of chitosan can be utilized for immobilization of other enzymes;
- determined urea detection limit at 10^{-4}M, which is in two orders below risk values for a healthy human;
- integration with other biomedical devices, *e.g.* in catheter tube (4 mm diameter tested).

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