Development of a LIG based-electrochemical sensor and a SERS-LFIA for sensitive detection of residual antibiotics in milk.

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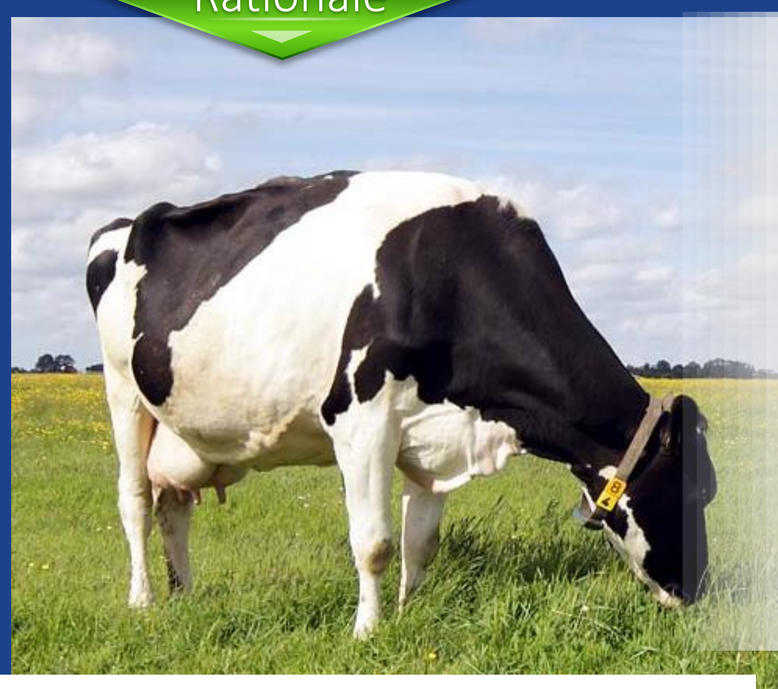
Rationale



Motivation

Antibiotics have been life-changing and often life-saving for both human beings and animals but Antimicrobial Resistance (AMR) is becoming a threat to human health because of overuse of antibiotics in agriculture, livestock farming, pharmaceutical industries and aquaculture.

Residual antibiotics have been found in soil, water, food.

In particular, for dairy farming, their presence in milk could be problematic for both human health and dairy production.

Impact

Point-of-care testing are really important for monitoring on field. They have a range of advantageous features including low cost, practicability and miniaturization.

For the farmers:

- ✓ Immediate knowledge of antibiotics presence in their milk
- ✓ Immediate knowledge of how to treat that milk.

Keywords: electrochemistry; laser-writing technique; antibiotics; point-of-care; milk; LFIA-SERS

Introduction

Antimicrobials, such as **Antibiotics**, are widely used for the treatment of bacterial infections of both human beings and animals.

Approximately 88% of veterinary drugs used in Ireland are made of older drug classes including tetracyclines, penicillins, aminoglycosides and potentiated sulphonamides.

The excessive use of this drugs can bring to a potential risk to human's health and ecological environment through the food chain and the ecological cycle.

There are many issues related to the use of antibiotics in dairy cows:

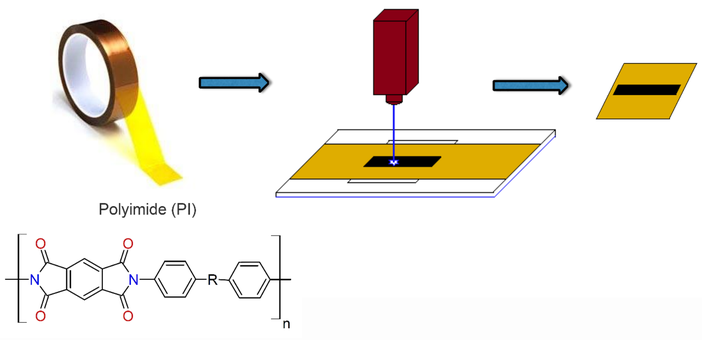
- Standard withdrawal times do not ensure that milk is drug-free. For example penicillin (withdrawal time 72 hours) can persist as long as 18 days in milk.
- The prophylactic use of antibiotics increases the risk of Antimicrobial Resistance (AMR) i.e. could cause the shift in resistance pattern of microbial population in human intestinal tract.
- 5-10% of population is hypersensitive to penicillin or other antibiotics present in milk even below the allowable level.
- Even small concentrations of antibiotics interfere with milk processing plants. They inhibit starter cultures for cheese and yoghurt production and change the acid and flavour production associated with butter production.

Background

Current methods for determination of residual antibiotics in milk:

- **Microbial Inhibition Screening Methods** — Fast, inaccurate
- **Analytical Instrument Detection Methods** (i.e. HPLC) — High sensitivity and specificity. Quantitative. Costly. Requires trained personnel and lab environments. Time consuming. €
- **Immunoassay Methods** (i.e. ELISA) — Accurate, selective and sensitive. Time-consuming, costly and requires trained personnel €

Electrode Materials

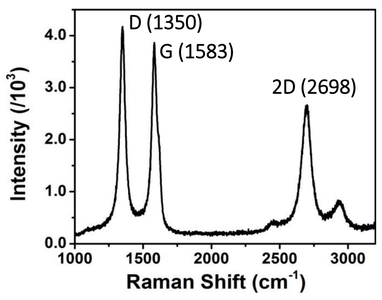


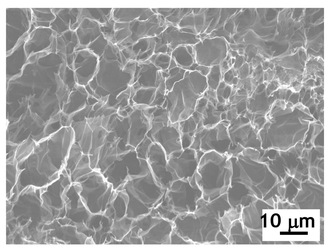
Schematic diagram of Direct Laser writing on Polyimide surface

Laser Induced Graphene (LIG)

- 3D, Porous, Conductive graphitic structures
- Ambient synthesis conditions
- Direct Laser writing provides design Versatility
- Low cost precursors offers bulk scale productions

Morphological Characterization

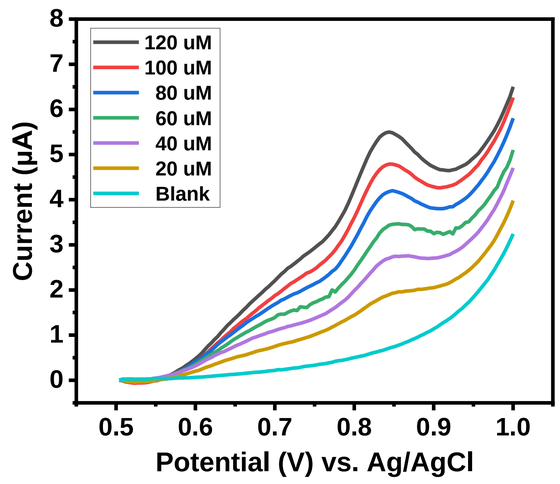




SEM Image of LIG

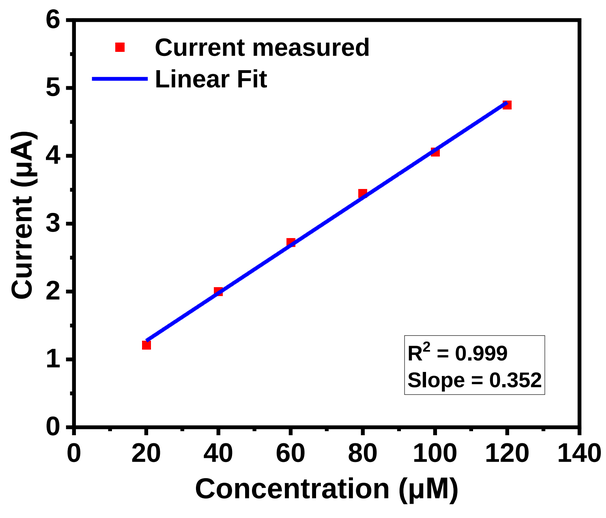
- Raman spectroscopy of LIG shows the formation of a graphite-like carbon morphology with the presence of D, G and 2D peaks with full width at half maximum in typical range of graphite-like carbon.
- Scanning electron microscopy shows the line microstructure with a regular and flaky structure showing high porosity and high surface area of the fabricated structural morphology.

Electrochemical Detection



Differential Pulse Voltammetry (DPV) for the detection of Tetracycline Hydrochloride in 0.1 M PBS

- DPV results show changes in peak currents at 0.848V for different concentrations of Tetracycline Hydrochloride using Laser induced graphene made on polyimide surface as electrode material.

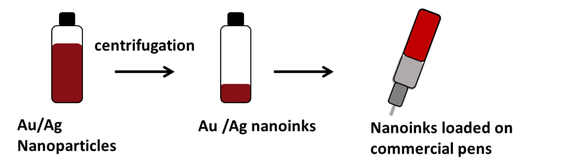


Calibration curve for the detection of Tetracycline Hydrochloride in 0.1 M PBS

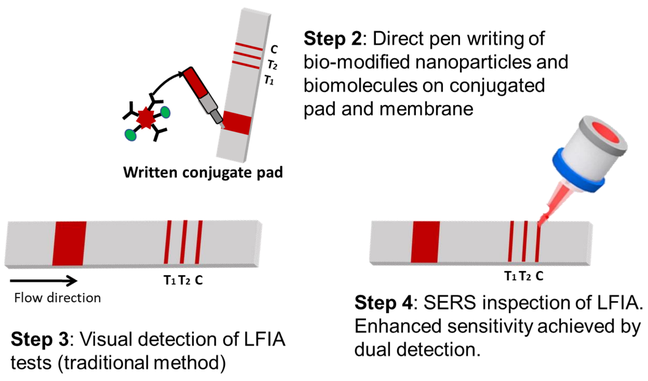
- Electrochemical responses are plotted against the concentrations of tetracycline hydrochloride to get a calibration curve for the concentrations ranging from 20-120 µM and it has been to have the sensitivity of 3.52 x 10^5 µA L mol^{-1} and 0.904 µA L^{-1}.

Electrode Materials	Conc. Ranges (µM)	Sensitivity (µA L mol^{-1})	LOD (µmol L^{-1})	Media
Bare LSGE	20 – 120	3.52 x 10^5	0.904	PBS (pH 7.4)

LFIA-SERS



Step 1: Direct pen writing of Au or Ag inks on conjugate pad

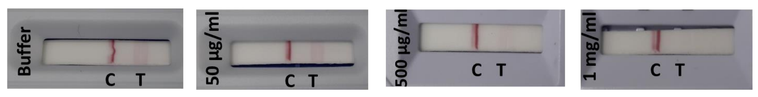


Step 2: Direct pen writing of bio-modified nanoparticles and biomolecules on conjugated pad and membrane

Step 3: Visual detection of LFIA tests (traditional method)

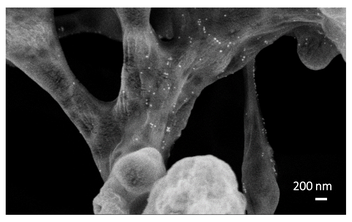
Step 4: SERS inspection of LFIA. Enhanced sensitivity achieved by dual detection.

Lateral Flow ImmunoAssay



Lateral flow immunoassays with Test line (T) and Control line (C) written by fountain pen with a fine nib: T=0.5 mg/ml Penicillin-BSA, C= 0.5 mg/ml AbvsMouse. Different concentration of antibiotic **Penicillin G** have been used in buffer to observe the competition between the antigen deposited in the test line and the antibiotic in solution.

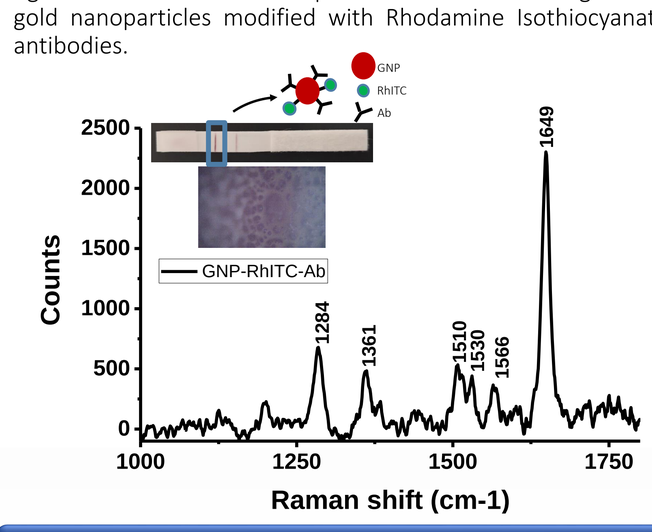
These pictures are related to preliminary results obtained at high concentration of antibiotics. For the purpose of the project, more studies will be done to reach a good sensitivity.



SEM images of LFIA line after the test where is possible to observe the nanoparticles in the nitrocellulose membrane.

Surface Enhanced Raman Scattering

Laser wavelength of 514 nm was used as excitation source. The figure below shows Raman spectrum of LFIA line using the SERS of gold nanoparticles modified with Rhodamine Isothiocyanate and antibodies.



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