Developing a self regulating control system for intravenous drug administration -- using aminoglycosides as an example

Project Members

Primary Contact	**Secondary Contact**
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Primarily contribute to the design and programming of the pump and help with the development of the sensor.	Primarily contribute to the design and programming of the pump and help with the development of the sensor.

Summary
Our goal is to put together a sensor-pump system which would be able to adjust drug output of aminoglycosides based on real time sensing of drug in plasma. This would involve several stages, the first being the development of a functional sensor over the appropriate therapeutic range of concentrations. Similarly, a device will be designed which can attach to current hospital drip bags to control output and have configurable parameters for information such as normal drug clearance, volume of distribution, concentration of drug being delivered, and so on. The software to calculate the appropriate output will also be written.

Problem being addressed
Drugs with a low therapeutic index or coping with patients with altered clearance due to situations such as liver or kidney failure can make the administration of the right amount of a drug difficult. Current monitoring procedures often involve laboratory tests which have an inherent delay as a result of logistic issues. As a result, patients may have plasma drug concentrations that are outside of the therapeutic window temporarily, which may increase the risk of side effects or ineffective treatment.

Outcomes and Benefits
It is hoped that developing such automated delivery systems will be able to simplify drug administration and reduce errors. This will be very useful in the future of personalised medicine, where it would be especially appropriate in patients with multiple comorbidities, that do not conform to the rest of the population by which the current system of drug administration is based on. In the process we also hope to develop a cheap and simple way to create an effective microfluidic device, which could also be used for many other purposes.

Biological Systems to be used
Electrochemical-aptamer based (E-AB) biosensors have been chosen for the sensor design as there are established designs available, have appropriate sensitivity, specificity, and dynamic range, and allows for easier interfacing with electrical devices. This would then be picked up, analysed and output to the pump by a control device.

E-AB sensor design
Electrochemical Aptamer based sensor(E-AB). The design and development of the E-AB sensor will need to be the first phase of the project. Ideally it should be a microfluidic device to enable portability and hopefully reduce cost. We will be researching to design and develop a simple microfluidic sensor chip which will need several components. We will first need to fabricate the sensor through attaching the aptamer to the gold leaf, following an immobilisation protocol[1]. Then, a platform will be designed for fluid flow, as detailed in diagram below. The gold leaf would be cut into the shape of the electrodes before being layered onto the platform.

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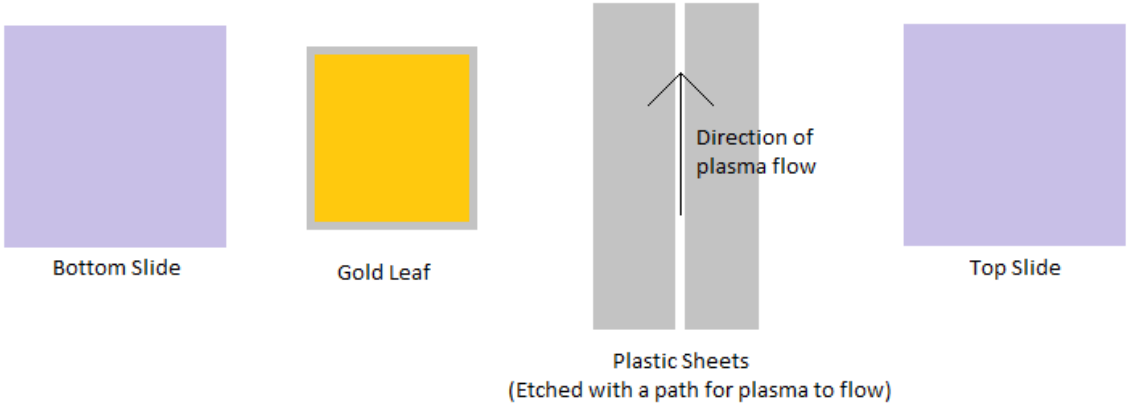

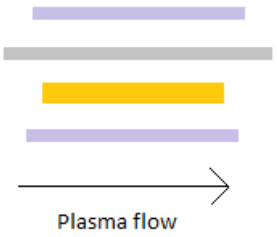

Top Slide (Microscope slide)

Plastic Sheets (2 or more sheets sandwiched together for thickness)

Gold Leaf (for the electrode design to be printed from)

Bottom Slide (Microscope slide)

Epoxy will be applied to areas not covered by material between the top and bottom slide to hold the layers together.

A shortlist of materials can be found in appendix but the design is not confirmed

Control device design

The control device will have

- Potentiostat to power and read input from the E-AB sensor
- Pump to provide fluid flow
- Chasis
- Computational power and software to process sensor data and modulate output
- Actuator to control drug output (potentially as an add on to current hospital drip bags)
- Modular sensor design to enable swapping out of sensor component

A shortlist of materials can be found in appendix

References

1. Aptamer immobilization protocol - http://www.nature.com/nprot/journal/v2/n11/full/nprot.2007.413.html
2. Tobramycin aptamer sequence - http://www.mdpi.com/1424-8220/15/4/7754/htm
3. Microfluidic electrochemical sensor design - http://microfluidics.utoronto.ca/papers/c4cs00369a.pdf
4. Electrochemical Biosensor Principles and Architectures - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3663003/
5. Real Time Aptamer based biosensor design - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4010950/
6. Cheap Potentiostat design - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3172209/
7. Sputtering - http://www.sciencedirect.com/science/article/pii/S1388248116301102
8. Integrating electrodes into microfluidic chip - http://www.microfluidicsinfo.com/electrodesimt.pdf
9. DIY Microfluidics - http://www.science-practice.com/blog/2015/01/29/low-tech-microfluidics/

Appendix - Materials List

Biosensor[1]

Material	Quantity	Estimated Cost	Source
Tobramycin aptamer	?	Quote needed	Biosearch Technologies
3d printed microfluidic device	?	Quote needed	Media Studio (Addenbrooke's Hospital)
Phosphate Buffered	1	£11.68/500ml	Fischer Scientific

Material	Quantity	Estimated Cost	Source
Tobramycin aptamer	?	Quote needed	Biosearch Technologies
3d printed microfluidic device	?	Quote needed	Media Studio (Addenbrooke's Hospital)
Phosphate Buffered Saline pH 7.2	1	£11.68/500ml	Fischer Scientific
Sodium Hydroxide(10M)	1	£35.30/L	Fischer Scientific
Potassium Chloride / Sulfuric Acid	1	£19.02/L	Fischer Scientific
1 um diamond suspension	1	?	Buehler
0.05um Gamma Alumina	1	?	Buehler
Microcloth	1	?	Buehler
Absolute Ethanol	1	£32.24/L	Fischer Scientific
6-Mercaptohexanol	1	£53.80/5ml	Sigma Aldrich
8 M guanidine-HCl	1	£106.50/100ml	Sigma Aldrich
Tris-2-carboxyehtyl phosphine hydrochloride	1	£68.50/10ml	Sigma Aldrich

Equipment needed

- Sonicator
- Potentiostat - Alternating Current Voltammetry
- Reference Electrode (Ag/AgCl)
- Platinum Wire
- Dark room

Potentiostat[6]

Board	Part Number	Maker	Purpose
1	ATXMEGA32A4-AU	Mouser	MCU
1	TLC2264ID	Mouser	Quad Op Amp
1	DG612AEY-T1-E3	Mouser	Quad SPST Switch (4 NO)
1	FT232RL	Mouser	USB-UART
1	FAN2501S33X_Q	Mouser	Linear Regulator
1	EADOGM163EEA	Mouser	16x3 Display
1	SKQUAAA010	Mouser	4 direction switch
1	154-15322-E	Mouser	USB Mini Type B connector
2	C0805C103K1RAC7210	Mouser	103 (0.01uF)
1	C0805C563K5RACTU	Mouser	56nF
7	C0805C104M5UAC7210	Mouser	104 (0.1uF)
3	C0805C105J4RAC7025	Mouser	105 (1uF)
4	GRM219R60J106KE19D	Mouser	10uF
2	GLCR2012T100M-HC	Mouser	10uH
2	MCB0805F400PT-T	Mouser	Ferrite Bead
12	22-28-5020	Mouser	PDI Header Pins + Cell
1	TNPW080533K0DEEA	Mouser	33kohm (0.5%, 1/8W)
2	TNPW080510K0DEEA	Mouser	10kohm (0.5%, 1/8W)
1	PFC-W0805LF-03-1653-B	Mouser	165kohm (0.1%,.1W)
2	ME-100	Mouser	jumper
1	PCB	4PCB	PCB Board
Case			
1	1593YGY	Mouser	Case
1	101-0110-EV	Mouser	12x12 mountain switch
Misc			
1	TPS61097-33DBVT		DC DC Converter
1	SRB22A2FBBNN	Mouser	Power switch
1	12BH324A-GR	Mouser	Battery Holder
1	AT25DF041A-SH-B	Mouser	Flash Chip

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Method

3rd phase

- Software to be able to predict output based current plasma levels (comes with the hardware, still need to be programmed)

4th phase

- Test the system out with more complicated drugs and develop additional modules to handle it, such as for drugs with high Vd, Slow Distribution, Low blood/gas and High Tissue/Blood coefficients

Beaker of plasma, with sensor to detect levels and feedback to the effector to increase or decrease input of drug into the bloodstream.

Further checked with the use of plasma switching to mimic clearance in real humans.

Proof of concept

Outcome and Benefit

To be able to simplify the detection of the drug levels in plasma and to be able to generate a constant feedback loop allowing the drug levels to be tailored to the patient's clearance of the drug.

1st phase

- Low Vd(~3L), Low therapeutic dose (***aminoglycoside***, cardiac glycosides, Chemotherapeutics), Rapid redistribution, High Blood/Gas, Low Tissue/Blood coefficients
- What makes a good biosensor (sensitivity, etc.)
    - Transduction system to detect the drug in question

Things to do (Saturday)

MAIN - Choose an antibiotic and read up on it - see the different methods of sensing drug levels

What range do we need biosensor to be sensitive over - read on this

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Antibiotics

Water soluble (very low Vd, just plasma [plasma proteins]) - Daptomycin

(Low Vd, including ECF) - Beta Lactam Anitbiotics and
Aminoglycosides(Gentamicin/tobramycin)

(High Vd) -- synercid, macrolide, FQ

Lipid Soluble (extremely high Vd, sequestered in tissues) -- Azithromycin

Vd for drugs increased with sepsis and fever

Elimination
Aminoglycosides (gentamicin/tobramycin) - mainly by glomerular filtration

High levels in blood may exacerbate renal failure. Bactericidal effect is concentration dependant

Sensing
Therapeutic Drug Monitoring
Currently done by many different professionals, e.g. physicians, clinical pharmacologists, clinical pharmacists, nurses, medical laboratory scientists, etc

2 methods

Priori -- Based on subpopulations and desired clinical endpoint to predict dose regimen
Posteriori -- pre analytical, analytical and post-analytical phases to determine dose regimen by detecting ACTIVE and TOXIC forms of the drug.

Sensors

(are enzymes difficult to work with?)

+transcutaneously
+intravenously (most probably this for us, due to simplicity)

1. Electrochemical RNA aptamer based sensors - folding based electrochemical sensors, electrode bound RNA(DNA) based aptamer biorecognition element. When the 26 nucleotide RNA aptamer sequence undergoes large conformational changes, the sensor works better.

   Very good method. Testing used cell samples to get results. But it should be able to be used to detect in blood plasma. RNA aptamers have a higher binding affinity, but may be degraded by nucleases in the blood plasma. DNA aptamers have a lower binding affinity, but may be sufficient. RNA sensors appear to degrade only after a few days. Can be solved with a microfluidic system that protects the RNA sensor from the nucleases.

   Affinity range -- 200nM to 42 μM (tested with tobramycin)

2. Amperometric biosensors - enzyme specificity and transduction of biocatalytic reactions into current signals. This can be single use, intermitent use or continuous use.

- Oxidase enzyme (H202 to generate electrons)
- Dehydrogenase Enzyme (NADH to generate electrons)

   Performance depends on the enzyme imobilisation techiques. Problems of fluctuating based on other substances in bloodstream and oxygen levels can be avoided by transferring electrons directly from the

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Used for Paracetamol and Chlorpromazine

Range for sensitivity necessary

Therapeutic range -- 2–6 µM (4–10 µg/ mL)
Tobramycin and kanamycin -- hyperbolic saturation curves (single site binding), Kd = 319 and 281 µM (These are lower when in solution phase) Kd = 12 nM to 13.2 µM

Gentamicin -- Biphasic curves with decreased biosensor current at low concentrations but increased current at higher concentrations

Dosage predictions with software
I have a feeling we might need a simple self learning neural network to be able to personalise it to each individual. But this doesn't seem like a priority right now.
The main important links

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3675903/

https://www.ncbi.nlm.nih.gov/pubmed/24377296

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3082472/

https://www.ncbi.nlm.nih.gov/pubmed/972270

http://www.sciencedirect.com/science/article/pii/S0731708598000569

Microfluidic Chip Design
Standard Venipuncture needle is 21 gauge : 514 micron diameter : 2.075 * 10^5 micron^2 cross section area

Reynold's Number =

u = velocity of fluid - si unit (m/s)
L = characteristic linear dimension - si unit (m)
v = kinematic viscosity - si unit (m^2/s)

Laminar flow defined as reynold number < 1000
Whole blood kinematic viscosity at 37 deg C ~ 2.65 mm^2/s = 2.65 * 10 ^ -6 m^2 / s
Linear dimension should be roughly the same as the 21 gauge meaning 514 micron diameter so around 5.14 * 10 -4 m

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So in order to maintain a reynolds number below 1000 we would need to have flow rates below 5 cm/s.

Assuming a **rectangular cross section area** for the microfluidic device, if we take the width of the channel to be **514 microns**, the height needs to be 403.70 microns or around **400 microns**. The thickness of a piece of paper or transparency is around **100 microns**, so we may need to

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Assuming a **rectangular cross section area** for the microfluidic device, if we take the width of the channel to be **514 microns**, the height needs to be 403.70 microns or around **400 microns**. The thickness of a piece of paper or transparency is around **100 microns**, so we may need to stack 3 or 4 sheets accounting for glue adding thickness and thickness of the imprinted electrodes in the channel. Ideally we should have a micrometer screw gauge to measure this. To ensure the width of the channel is about 514 microns, we could devise a **wedge out of plastic sheets and glue to about 500 microns** and then when we etch make sure it fits inside the etching? I think we should enquire about the 3D printing process as well, once I get an AutoCAD design file done maybe we can get a quotation from addenbrookes and compare the price to doing it with the gilding.

Another issue to consider is the printing of the electrodes onto the chip. From what I read the counter electrode needs to be larger than the working by maybe around 10 times. A standard glass slide length is around 75mm so maximally if the working electrode has a length of 7.5mm then the working electrode needs to have a length of 75mm. However we need to leave some space for reference electrode as well, so maybe if we have a 5mm length working electrode and a 5cm length counter electrode, then around 2cm is leftover for the reference. We probably need to read up more on electrode design because the position and shape etc of electrodes can affect as well. **But I was thinking of printing the reference and counter electrodes on one glass slide (top) and working on the other (bottom)** so that when we have to apply the aptamer immobilisation protocol we can do it to working electrode without worrying about adding them to the counter and reference. However, if we use platinum leaf for the counter and reference maybe the reaction won't affect them as well. Nevertheless I think if we separate the working and counter+reference electrode its better for the system also, like the current passing in the counter electrode might affect other electrodes not sure.