Perfecting Bacterial Tumor Treatment using Microfluidic Bioreactors

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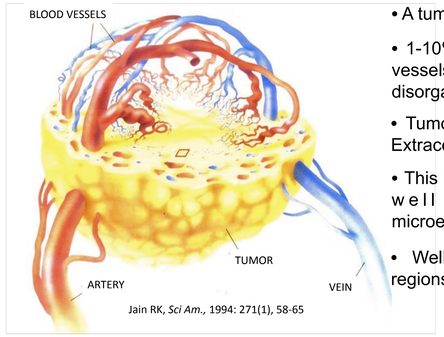
Tumor Physiology

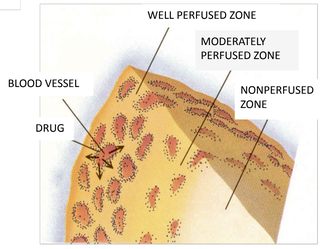


Jain RK, *Sci Am.*, 1994: 271(1), 58-65

- A tumor is a cluster of rapidly growing cells.
- 1-10% of the volume is occupied by blood vessels. In tumors these are highly disorganized.
- Tumors are characterized by an extensive Extracellular Matrix.
- This unique physiology leads to spatially as well as dynamically varying microenvironments within the tumor.
- Well-perfused as well as non-perfused regions exist within the tumor.

What are the limitations of current cancer chemotherapeutics?

- *Ineffective penetration*
- *Ineffective targeting*
- *Excessive Toxicity*
- *Recurrence and Metastasis*



Jain RK, *Sci Am.*, 1994: 271(1), 58-65

Objectives

- ***Develop an in-vitro model of 3-dimensional tumor tissue that allows observation of long term tumor response to therapeutic bacteria (an "artificial tumor").***
- ***Using this model, quantify apoptosis induced by therapeutic bacteria in tumor tissue over physiologically relevant time scales.***

Why microfluidic ?

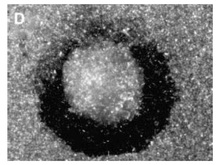
- High bacterial growth rates

Rapid proliferation of bacteria in medium surrounding the tissue has limited the time span of earlier *in-vitro* experiments. A continuous flow-through system would enable significant increase in time span of the experiment.

- Drug pharmacokinetics

Batch *in-vitro* systems do not enable us to subject tissue to desired drug/ bacteria pharmacokinetics. Continuous flow-through systems provide us with an opportunity to do so.

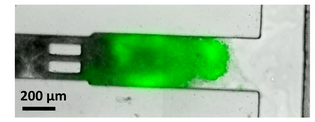
Microfluidic lab-on-a-chip type devices provide the perfect platform for creating continuous flow-through bioreactors.



Excessive bacteria concentration in medium surrounding cylindroid after 22 hours of inoculation.
Kasinskas RW and Forbes NS. *Biotech. and Bioeng.*, 2006, 94:4, 710

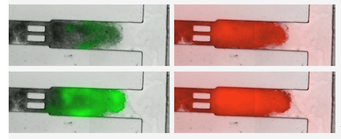
Bacterial Experiments

- Artificial tumors thus formed were subjected to 1-hour long plugs of a constitutively GFP-expressing strain of *Salmonella Typhimurium* bacteria SL1344. Bacteria formed colonies preferentially in the tumor tissue without significant rise in the medium bacteria concentration in experiments lasting longer than 40 hours.



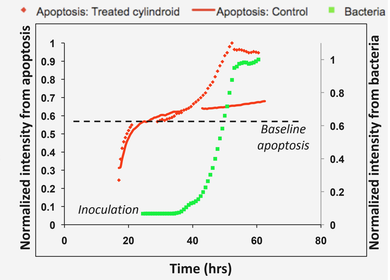
Accumulation of GFP-expressing bacteria in tumor tissue without increase in medium concentration. Figure shows overlay of acquired transmitted light and fluorescent images.
= bacteria

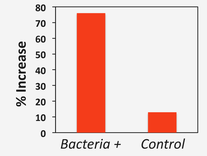
- Accumulation of bacteria was found to induce apoptosis within the tissue. Apoptosis was quantified by a fluorescent dye that stained active caspase-3.



= bacteria
= apoptosis

Bacterial colonization induced apoptosis

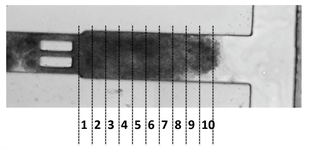


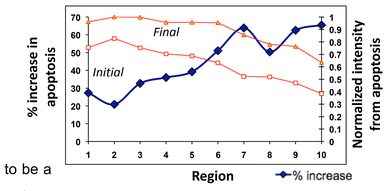


Percentage increase in apoptosis over entire tissue from baseline levels

- Microenvironment-dependent cytotoxicity

Further insights can be gained into the mechanism of bacterial action by quantifying apoptosis induced by bacteria as a function of location within the tissue. The tissue was divided into 10 regions of equal width and the relative increase in apoptosis over a fixed period of time was evaluated.





Percentage increase in apoptosis over a period of 17 hours

- Percentage of Increase in apoptosis was found to be a function of location within tissue. A maximum percentage increase in the induced apoptosis may be predicted to exist in region 7.

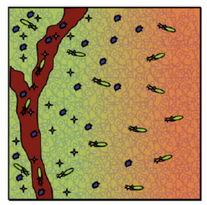
Why Bacteria ?

- *Active* as opposed to *Passive* Transport

Chemotherapeutics are often limited by their ability to penetrate within the tumor as they rely on passive diffusion. Therapeutic bacteria possess the ability to actively transport deeper into the tumor tissue.

- Genetic Modifiability

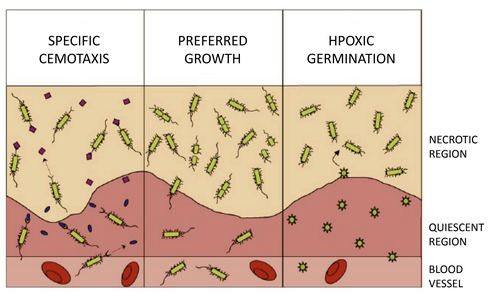
Therapeutic bacteria can be genetically modified to increase their affinity towards desired tumor regions, thus providing a targeting mechanism. Deleting the ribose/galactose chemoreceptor for instance, leads to accumulation within therapeutically resistant regions. They can further be engineered to express desired cytotoxins, and control their release.



POOR PENETRATING DRUG
DEEP PENETRATING DRUG
THERAPEUTIC BACTERIUM

Walsh et al., *Lab Chip*, 2009, 9, 545-554

Bacterial Tumor Targeting Mechanisms



St Jean and Zhang et al., *Curr Op Biotechnol,* 2008, 19, 511-517

- **Specific Chemotaxis**: Bacteria with specific chemoreceptors sense complimentary chemicals and actively swim towards regions rich in those chemicals.
- **Preferred Growth**: Post extravasation, bacteria find certain regions within the tumor favorable for proliferation as shown by dividing cells.
- **Hypoxic Germination**: Spores of strict anaerobic bacteria extravasate into the tumor and germinate specifically in hypoxic regions of the tumor.

"*Tumor-on-a-chip*" - The Design

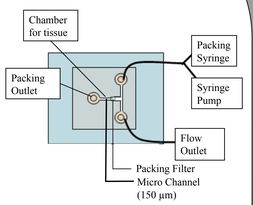
- Multicellular tumor spheroids are excellent models of in-vitro tumor tissue. They mimic the microenvironments within tumors and contain proliferating, quiescent, as well as necrotic regions.

➢ **Step 1: Packing**

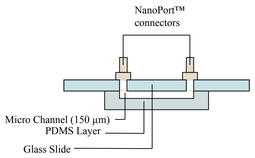
Spheroids were directed by flow and constrained into micro-chambers on the chip. The packing outlet was open while the flow outlet was closed.

➢ **Step 2: Equilibration, Growth and Treatment**

Once in place, the tissue was subjected to mild perfusion of medium from one side. This created nutrient gradients in the tissue away from the channel. Tissue can be grown in this fashion for long term experiments. The packing outlet was closed while the flow outlet was open.

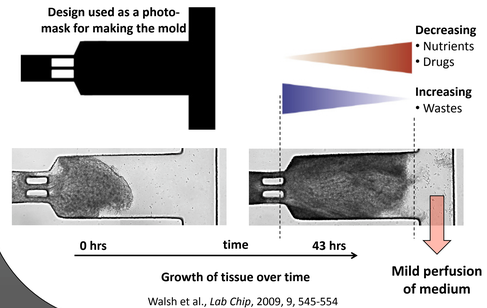


Top View



Cross Section

Walsh et al., *Lab Chip*, 2009, 9, 545-554



Walsh et al., *Lab Chip*, 2009, 9, 545-554

- Photoresist soft lithography was used for fabrication.
- All chips were made of PDMS and attached to a glass slide by oxygen plasma treatment.

Summary and Conclusions

- Therapeutic bacteria have the potential to overcome the limitation of current cancer chemotherapeutics.
- Artificial tumors that allowed observation of long term tissue response to bacteria were created using photoresist soft lithography.
- Therapeutic bacteria accumulated in tumor tissue and significantly enhanced apoptosis.
- Time lapse microscopy allowed continuous monitoring of tissue for mechanistic studies.

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