Bacteria are Picky Homeowners

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BACKGROUND

Bacteria can exist as **planktonic** single cells or as part of a community called a **biofilm**.

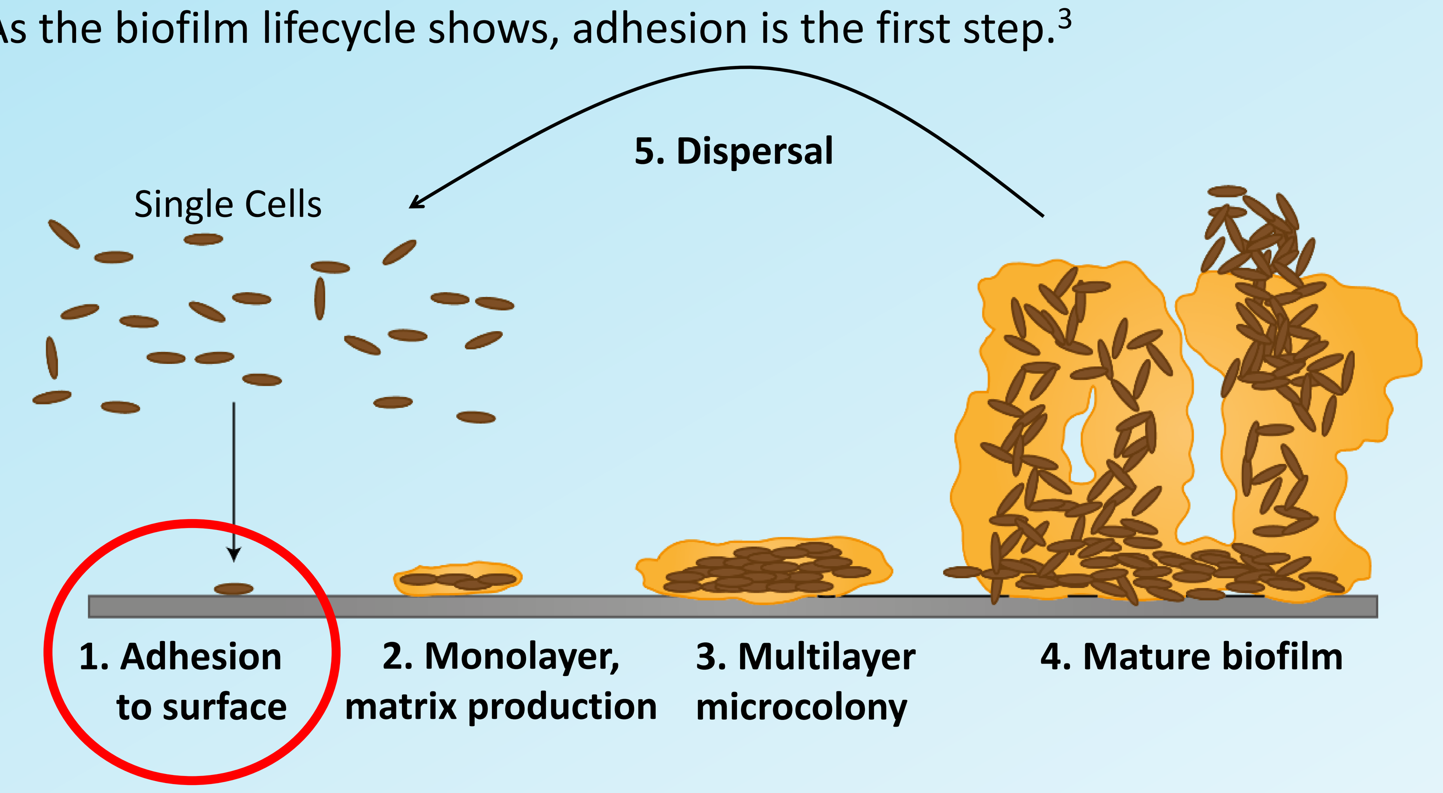
Biofilms are not just clumps of cells, but complex biological systems that are 10-1000x times harder to eradicate compared to single cells[1].

	BIOFILM	PLANKTONIC (SINGLE CELLS)
Antibiotic resistance	✓	✗
Protective matrix	✓	✗
High virulence	✓	✗

Unfortunately, biofilms can easily contaminate the surfaces of inserted medical devices like catheters, heart valves and stents.

This leads to **biofilm-associated infections** which cause over 500 000 deaths each year and cost $100 billion to global GDP to treat[2].

As the biofilm lifecycle shows, adhesion is the first step.[3]



Preventing this first step may be key to stopping biofilms!

It is well known that bacteria use extracellular appendages to detect, differentiate, and **show preference** to certain surfaces over others based on mechanical properties like roughness, thickness, charge, wettability, etc[3].

For example, on hydrophobic surfaces (which repel water), several studies have found that bacterial adhesion increases as surface stiffness decreases[4].

OBJECTIVE

However, the **interplay *between* wettability and stiffness on adhesion** has not been elucidated.

Therefore, this study explored the effects of surface **stiffness and hydrophilicity** (water-loving) on bacterial adhesion using *Escherichia coli*.

Will we see more bacterial adhesion on softer, wetter material? Will there be no difference?

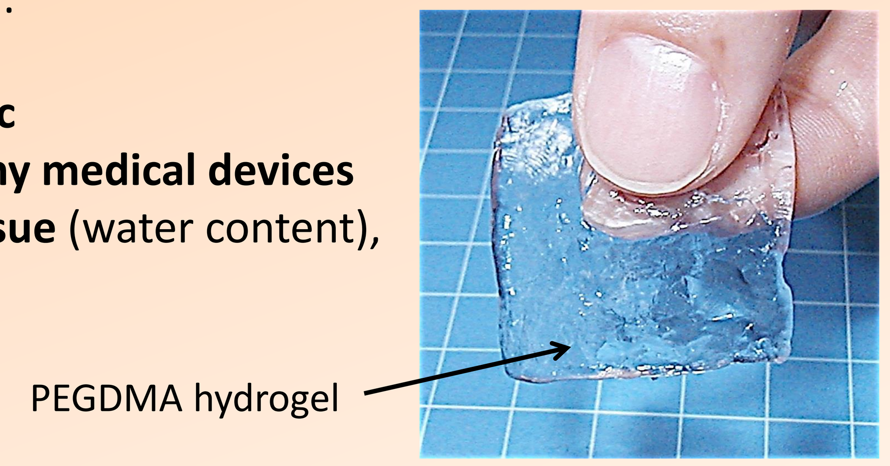
METHODS

The experimental design for this study consisted of the following steps:

1. Develop hydrophilic surfaces of varying stiffness
2. Measure physical properties of the surfaces (like stiffness and hydrophilicity)
3. Expose surfaces to *E. coli*
4. Measure and compare *E. coli* growth on the different surfaces

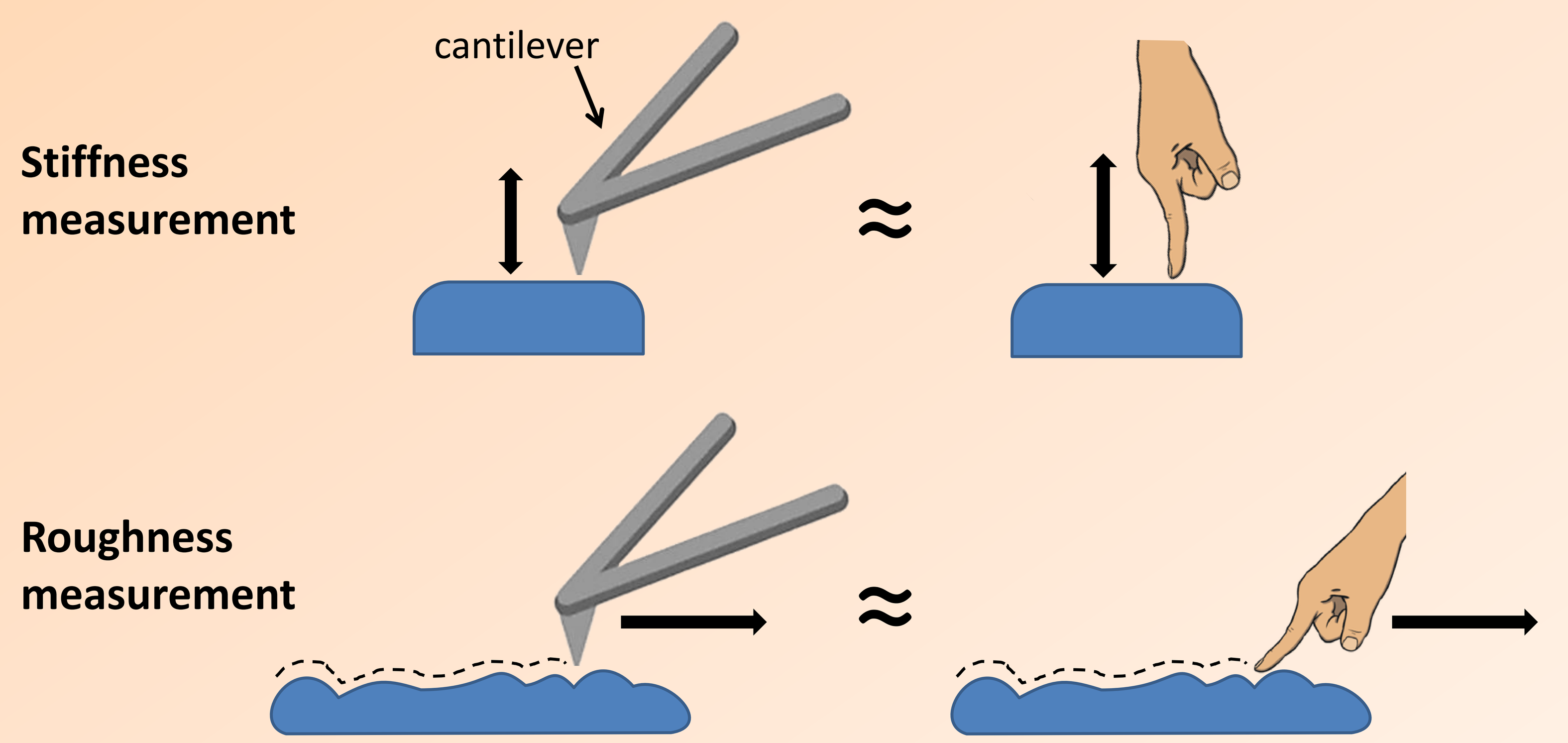
The material of choice to design the surfaces was poly (ethylene glycol) dimethacrylate (PEGDMA) because:

- It is almost perfectly **hydrophilic**
- It has **similar properties to many medical devices** (gel coating) **AND biological tissue** (water content), making it highly relevant[5]



An Atomic Force Microscope (AFM) was used to measure the stiffness and roughness of the surfaces.

The *cantilever* of an AFM can be thought of as a finger pressing on a surface to determine how hard it is. Similarly the cantilever can run across the surface to determine its roughness[6].



RESULTS

Hydrogel (Surface)	Stiffness (Young's Modulus, MPa)	Hydrophilicity	Roughness (R_q, nm)
Stiff (40% PEGDMA)	6.0 ± 4.0	~100%	5.94 ± 0.08
Soft (7.5% PEGDMA)	≤ 0.16 ± 0.04	~100%	6.20 ± 2.18

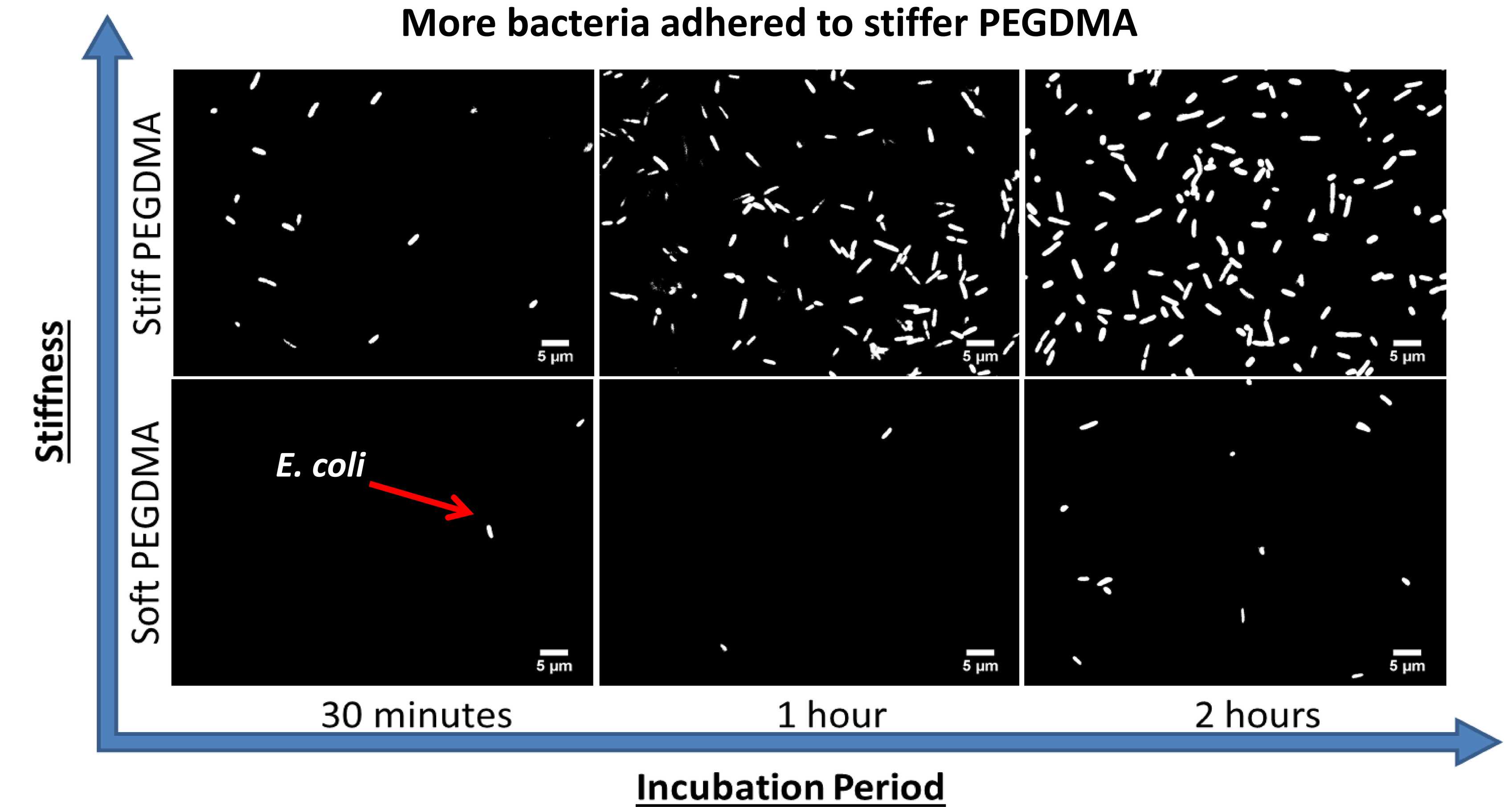
Stiffness is measured in units of pascals (Pa) and the stiff hydrogel was found to be approximately 38 times stiffer than the soft hydrogel.

Hydrophilicity was measured using a water drop test (not shown here) and both hydrogels demonstrated near perfect hydrophilicity as expected.

Roughness was measured to control for confounding variables. Both hydrogels were found to be equally smooth (~6nm), so it is reasonably presumed that this property did not account for any differences in bacterial adhesion.

Adhesion results were determined using confocal microscope imaging as shown below. At all time points, there was greater *E. coli* adhesion on the stiff hydrogels compared to the soft hydrogels. As mentioned, the opposite trend was observed in previous studies working with hydrophobic material[4].

More bacteria adhered to stiffer PEGDMA



DISCUSSION

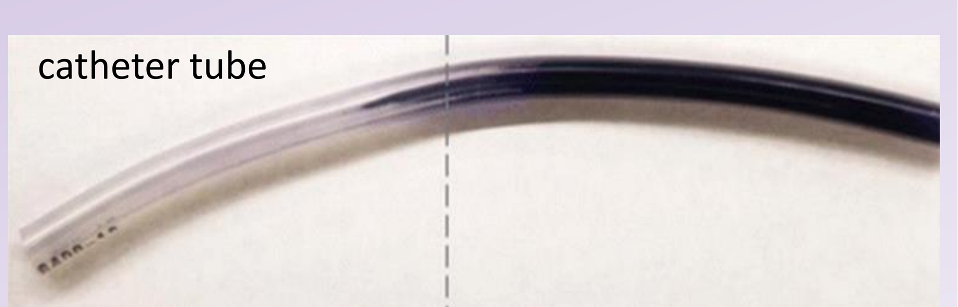
The two main conclusions from this study are:

- **On hydrophilic material, *E. coli* shows greater adhesion to stiffer surfaces**
- **A substrate's wettability plays a role along with stiffness in influencing bacterial adhesion**

This study promotes University of Toronto's Strategic Research Plan. It helped us **DISCOVER** that bacteria are less likely to adhere to stiffer substrates made of hydrophilic material. Accordingly, we can **PROMOTE** relatively simple changes in medical device manufacturing which can result in significant reductions in biofilm infections.

For example, we can **INNOVATE** new medical devices made of softer, hydrophilic material.

Alternatively, we can coat catheters and stents with soft, hydrophilic hydrogels to reduce bacterial adhesion.



Bacteria-free hydrogel coated side | Contaminated untreated side

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