ESTEC, 13th February 2017, Db124

SymSe Workshop on Biomaterials

Designed by Agata Kolodziejczyk, 2017

10.00 Introduction to biomaterials and bioprinting
11.00 Methods
12.00 Lunch
13.00 Manufacturing
17.00 Summary test

List of participants

1. Vincent Friebe, VU University Amsterdam
2. Hongyu Sun, VU University Amsterdam
3. Cees de Wit, VU University Amsterdam
4. Ilya Kolpakov, VU University Amsterdam
5. Stefan Tabernig, VU University Amsterdam
6. Albert These, VU University Amsterdam
7. Matt Harasymczuk, University of Warsaw
8. Ricarda Schroeder, Advanced Concepts Team, ESA
9. Kamil Adamczyk, Interclinic
10. Michal Adamczyk, Interclinic
11. Andjela Tomic, SymSe Team, Willem de Kooning Academy Rotterdam University
12. Ilfa Siebenhaar, SymSe Team, Willem de Kooning Academy Rotterdam University
13. Bram van Waardenberg, SymSe Team, Willem de Kooning Academy
14. Philippa Horgan, SymSe Team, Willem de Kooning Academy Rotterdam University
15. Sara Pavicics, SymSe Team Willem de Kooning Academy Rotterdam University
16. Susanne Vos, SymSe Team, Willem de Kooning Academy Rotterdam University
17. M.A.C. Nerrings, SymSe Team, Willem de Kooning Academy Rotterdam University
18. Remi Veldhoven (arrives later…14.00), SymSe Team

Perhaps / not confirmed
19.Sonja Stedt, Willem de Kooning Academy Rotterdam University
20.Ermi van Oers, Willem de Kooning Academy Rotterdam University
21.Thijs Meindertsma, Willem de Kooning Academy Rotterdam University
22. River Diephuis, Willem de Kooning Academy Rotterdam University

BIOMATERIALS

A **biofilm** is any group of microorganisms in which cells stick to each other and often these cells adhere to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm extracellular polymeric substance, which is also referred to as **slime** (although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium.
Microbial systems are inherently complex. A key challenge is to understand the biotic and abiotic interactions of microbial systems to the endpoint of prediction (Prosser et al., 2007). However, many microbial systems found in nature are difficult to manipulate experimentally and have under-described diversity as well as many unknown or intractable functions (Jessup et al., 2004). On the other hand, many model laboratory systems, in microbiology and other fields, are arguably over-simplified, maintained in controlled conditions generally unrealistic to naturally occurring systems (e.g. (Carpenter and Url, 2011)).

Kombucha

A kombucha culture is a symbiotic culture of bacteria and yeast (SCOBY), similar to mother of vinegar, containing one or more species each of bacteria and yeasts, which form a zoogleal mat known as a "mother." The cultures may contain one or more of the *yeasts Saccharomyces cerevisiae*, *Brettanomyces bruxellensis*, *Candida stellata*, *Schizosaccharomyces pombe*, and *Zygosaccharomyces bailii*

The *bacterial component* of kombucha comprises several species, almost always including *Gluconacetobacter xylinus* (*G. xylinus*, formerly *Acetobacter xylinum*), which ferments alcohols produced by the yeasts into acetic and other acids, increasing the acidity and limiting ethanol content. The population of bacteria and yeasts found to produce acetic acid has been reported to increase for the first 4 days of fermentation, decreasing thereafter. *G. xylinum* has been shown to produce microbial cellulose, and is reportedly responsible for most or all of the physical structure of the "mother", which may have been selectively encouraged over time for firmer (denser) and more robust cultures by brewers.

The Kombucha Biofilm: a Model System for Microbial Ecology

Microbial systems are inherently complex and difficult to predict. A strong model system of naturally-occurring microbial consortia would provide opportunity to address key questions in microbial ecology. Kombucha is a traditional, fermented tea produced by a biofilm of yeast and acetic acid producing bacteria. Because it has well-defined functional products and is maintained easily at the laboratory-scale, kombucha has potential as a mixed-microbial model system. Kombucha microbial consortia are described using isolation and 16S 454 tag-pyrosequencing. Kombucha habours a dominant Glucoacetobacter population, and uncovered evidence for more resolved population-level diversity within the biofilm. Cell counts of the kombucha broth peaked at pH 3 on day 3 after innoculation, indicating that the acid concentration of the peak may provide a strong environmental filter for the microbes. The biofilm is heterogeneous in space, containing patches of yeast within a matrix of cellulose and segregated bacterial cells. Finally, tea origin and pH partially determine the rate of product evolution in kombucha broth, and tea may inhibit the growth of the consortia.

Kombucha is a beverage of fermented tea consumed traditionally in eastern Europe and Asia. To prepare kombucha, black tea leaves are seeped in boiling water, copious amounts of sugar (sucrose) is added, and the mixture is cooled to room temperature. Then a "mother" biofilm is placed into the tea; this biofilm is sometimes
called a symbiotic culture of bacteria and yeasts (SCOBY), and is comprised of acetic acid producing bacteria, ethanol fermenting yeasts, and a thick cellulose pellicle. This starter biofilm, or unpasteurized liquid from an active kombucha culture, is necessary to begin kombucha production. The biofilm floats at the liquid-air interface and grows vertically, increasing biomass with cellulose striations as the fermentation matures. After several days incubation at room temperature, the tea becomes a sweet and sour, naturally carbonated beverage because of microbial activities. Kombucha has been both sanctioned and advocated as a health beverage, with no consensus (Dufresne, 2000). However, kombucha consumption is gaining popularity worldwide, and there is much interest in scaling up the fermentation process to meet food industry demands (e.g. (Chen and Liu, 2000; Malbasa et al., 2006; Cvetkovic et al., 2008; Jayabalan et al., 2008)).
The general products of kombucha fermentation are **sugars and organic acids**. The yeasts convert sucrose to glucose and fructose during fermentation, as a byproduct of ethanol fermentation (Blanc, 1996). The acetic acid producing bacteria then convert fructose into acetic acid (which provides kombucha with its sour flavor) and glucose to gluconic acid. Accordingly, the pH of the enrichment drops to approximately 2.6 after, signifying the maturation of the beverage for consumption. If the fermentation is not stopped or slowed, the gluconic and acetic acid concentrations will continue to increase to levels of (4 g/100 mL, (Chen and Liu, 2000)), but the beverage will be intolerable for consumption because of a strong vinegar flavor. An additional byproduct of acetic acid production by bacteria is cellulose (Iguchi et al., 2000), which provides scaffold to the biofilm-associated microbes.Because the kombucha biofilm is a mixed eukaryotic-bacterial microbial community and has clear functions and products, it may serve as an excellent study system for microbial ecology. There has been identification of some of the yeast and bacterial species found in various SCOBYs. It was found that the species composition of kombuchas vary by biofilm origin, especially with respect to the associated yeast

Indeed, much of the basic microbial ecology of the kombucha system is undocumented. Yet ease of lab-scale culturing and manipulation (the biofilm grows well and quickly at room temperature with minimal specialized equipment), kombucha has potential as a naturally occurring, simple, and authentic model for microbial communities and their interactions. Here, I provide a first exploration of the utility of kombucha microbial community as a model system. Using a combination of classical microbiology with molecular tools,I describe temporal and spatial dynamics of kombucha, from inoculation to culture maturation. I also conducted an experiment to understand the influence of tea origin and pH on the microbial products of kombucha.

Manufacturing photosynthetic biomaterial – Practical part of the workshop

Materials

1. 20 gloves
2. Piments:

   -white pearl

   -gold pearl

   -transparent shapphire 15ml (0.5-3%)

   -transparent jadeit 15 ml (0.5-3%)

   -transparent amber15ml (0.5-3%)

   - pigment fosfor

3. Syringes

- 1ml U40 insulin with needle sterile – 10x

- 50ml 4x

- 100ml 3x

4. Scafolding support

- Polyurethane resin (Rencast FC Fast Casting): polyol:isocyanate (1:1), life (25C): 6-8 min., preparation(25C): 60-90 min.

- Thermoplastic polymer (elastic and hard): heat up 60C, model, cool down until hardness

- Plastisol Soft Plastic K5: plasticity in microwave: 30s + 30s + 20s + 20s after adding the pigment.

- Progel mass to forming: heat up 90C intensively stirring, melt, cool down until 45C
- Silicone (MM940): silicone:catalyst (100:5), hardness 37 Shore A, Viscosity 37 000 mPas
- Filler, stabilizer (DT-99), Microballoon 120
- PFA plates
- Agar Petri dishes

Photosynthetic Bioinks

1. Algae
2. Cyanobacteria isolated from lichen *Xantoria parietina*
3. Symbiotic algae from mosses
4. Mosses

Exercise: Design and then make your own photosynthetic biomaterial