

BACTERIAL SEQUESTRATION BY HYDROGELS

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Aim: To demonstrate the capabilities of a series of novel hydrogels to differentially sequester different bacterial populations and to hold them in a viable state or alternatively, to kill them.

Method: Sequestrations, as a function of time, by three hydrogels, of *S. aureus* and *Ps aeruginosa* were measured by the Live/DeadR BacLight™ bacterial viability assay in which viable bacterial cells fluoresce green, whilst non-viable cells fluoresce red. Viable colony forming units (CFU) were determined from calibration curves, obtained by mixing known populations of live and dead bacteria for each bacterial strain.

Results:Table 1: *Ps. aeruginosa* (% live bacteria)Table 2: *S. aureus* (% live bacteria)

Time/hrs	Gel A	Gel B	Gel C		Time/hrs	Gel A	Gel B	Gel C
0	35	0	0		0	0	52	0
1	0	45	53		1	0	24	0
2	0	0	0		2	0	<10	24
3	77	>100	0		3	0	0	0
4	>100	>100	0		4	0	<10	0
5	>100	>100	23		5	0	0	84
6	>100	>100	0		6	0	0	13

Discussion: Effect of the Hydrogels on *Ps. Aeruginosa*. In the case of Gel A, the initially attached bacteria appear viable, which is not the case with gels B and C, where cell death is complete. Over the following 2 hours, the bacteria attached to gel A are killed and no further attachment occurs. In the case of Gel B and, to a lesser extent, in the case of Gel C also, attachment occurs, which is subsequently followed by cell death. Over the longer time period, up to 6 hours, major differences in behaviour are observed, bacterial growth becomes quite rapid in the cases of Gels A and B. In the case of Gel C however, the attached cells are killed and a further attempt at attachment at 5 hours also results in cell death. The consequences of the different types of behaviour are that, in the case of Gel B and, to a lesser extent Gel A, strong attachment is occurring to the gels and the bacteria remain viable. In the case of Gel C, cell death occurs continuously and no viable bacterial colonies are established.

Effect of the Hydrogels on *S. aureus*. The bacteria are very quickly attracted to the surface of Gel B, but are subsequently killed over the following 6 hours. A similar pattern is observed in the case of Gel A, but not so clearly. In the case of Gel C, a pattern resembling that with *Ps. aeruginosa* is observed, cell death, followed by a repopulation, which is again followed by cell death.

Conclusion: It is clear that Gel C is effective at killing both strains of bacteria. A particularly interesting situation occurs however in the case of Gel B. Over the 6 hour period, *S. aureus* are initially very strongly attracted and then subsequently killed. Over the same period however, initially no attraction of *Ps. aeruginosa* occurs but, subsequently it is attracted and remains viable on the gel surface. With this particular gel it is therefore possible to separately determine the two different bacterial populations.