Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Document

A Mechanistic Study on Tumour Spheroids Formation in Thermosensitive Hydrogels: Experiments and Mathematical Modelling

X Cui[a], S Dini[b], S Dai[a], J Bi[a], B J Binder[b], J E F Green[b] and H Zhang[a]

[a] School of Chemical Engineering , University of Adelaide, Adelaide, SA, Australia 5005

[b]. School of Mathematical Sciences, University of Adelaide, Adelaide, SA, Australia 5005

Corresponding :Dr. Hu Zhang

Hu.zhang@adelaide.edu.au

1.

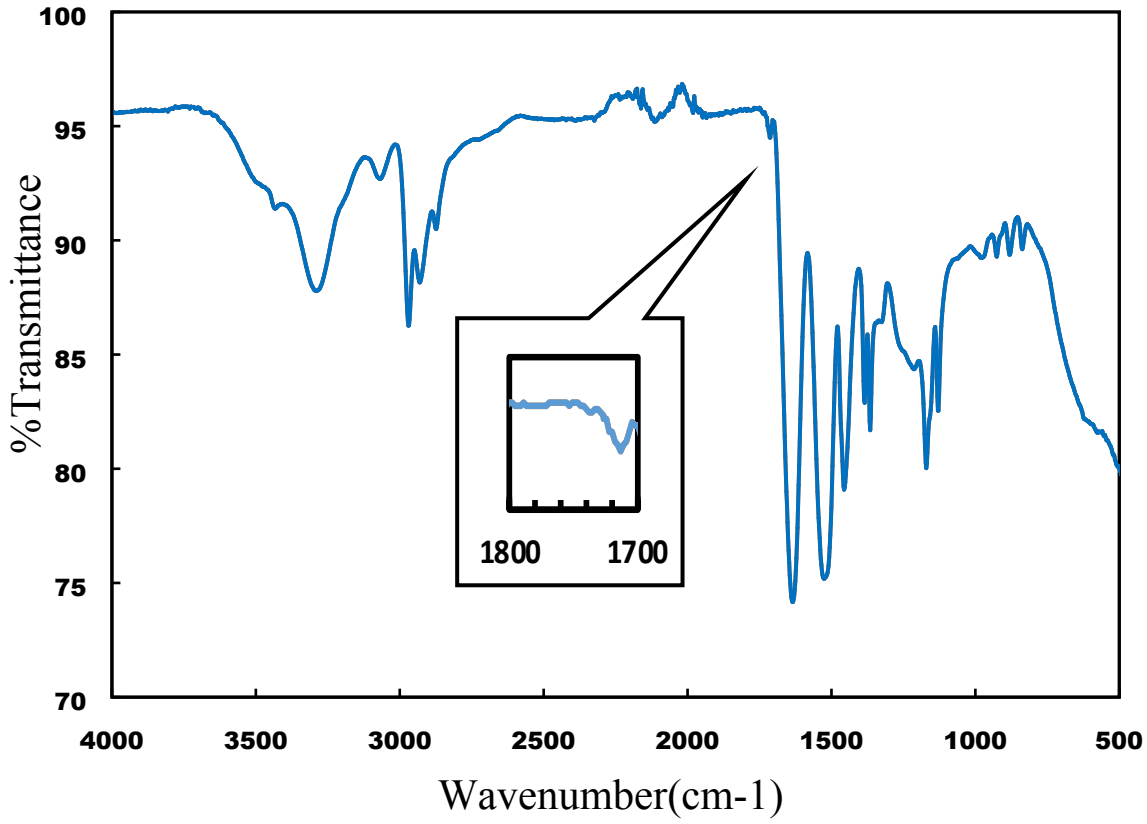

Figure S1 FTIR for P(NIPAM-AA)

2.

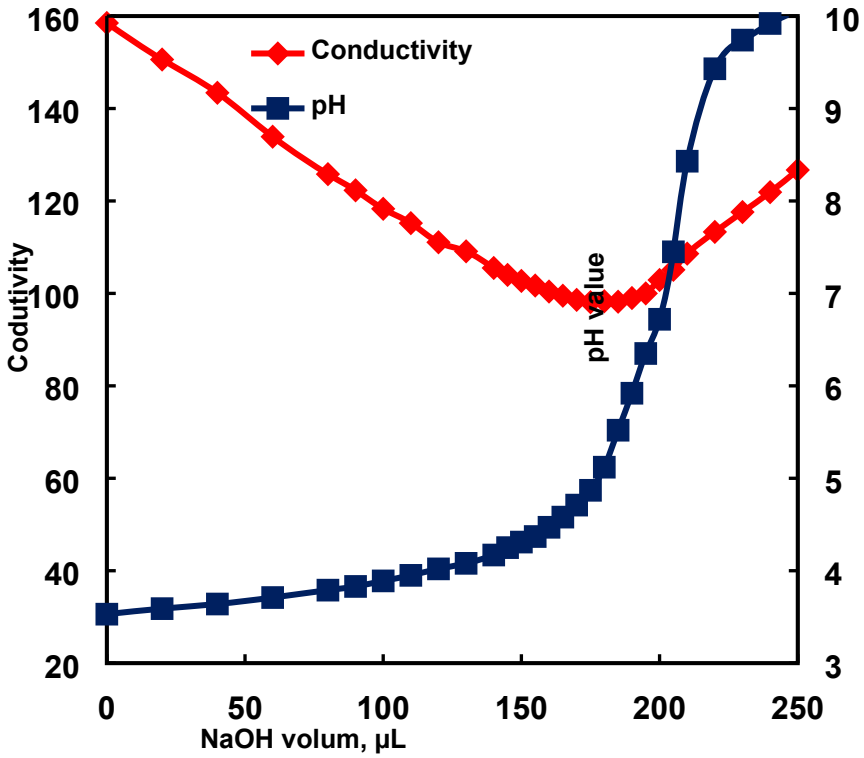

Figure S2 pH and conductive Titration of P(NIPAM-AA) microgel

3.

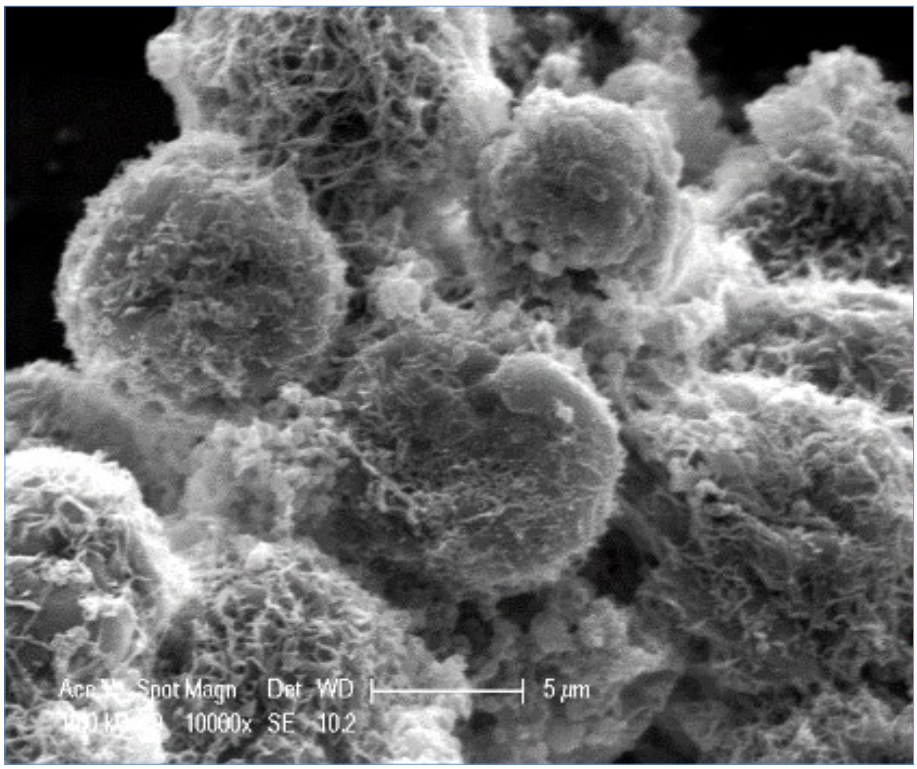

Figure S3. SEM of a HeLa spheroid showing cell to cell interaction

4.

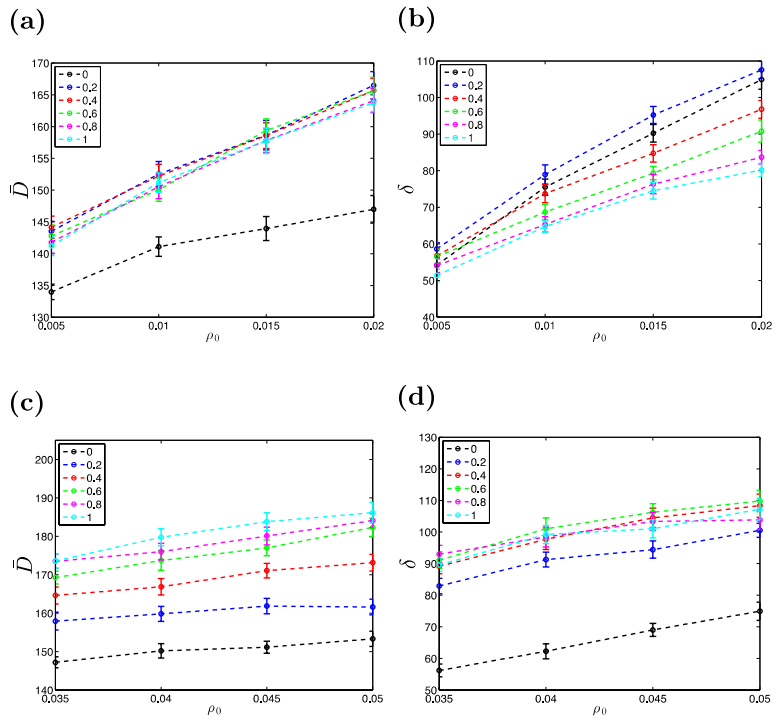

Figure S4 Parameter sweeping with a same range of Pm for the culture media. Standard deviation, δ, and average, $\bar{D}$, of cluster size are depicted for (a,b) microgel and (c,d) suspension. The curves are for Pm = {0, 0.2, 0.4, 0.6, 0.8, 0.1} with the colours as indicated in the legends of the graphs. Other parameters are the same as for the simulations of Figure 9

Here, we assess spheroid formation for a wider range of Pm values. This allows to analyse this process for culture methods with different characteristics than those of the experiments reported in this paper.

Figures S4(a) and S4(c) show that D increases with Pm (see also Figure S5(a) for further simulations of microgel). This is because when the cells are able to move freely, random movements help them to find clusters in their surrounding area and adhere to them. The simulations show that even cells in small clusters may find larger ones in their neighbourhood and attach to them. This leads to removal of some of small clusters and formation of bigger ones.

Figure S4(d) illustrates a big gap between δ for Pm=0 and δ for other values of Pm in suspension. This occurs due to the fact that cells die when they cannot move towards the other cells and adhere to them. Hence, few clusters remain after 21 days and this results in a lower standard deviation of cluster size. However, we note that this is not practically desirable. This figure indicates that there is no significant difference between the curves of δ for higher values of Pm.

The standard deviation of cluster size for microgels is quite different: δ reduces with Pm, see Figure S4(b). This means that a hypothetical medium allowing higher cell motility (whilst retaining the other features of microgels) might improve the uniformity of size of the clusters. However, this is not applicable to the experiments undertaken here as the parameter regime for those cases is quite different. However, these results give insights into how different culture methods might affect spheroid formation.

5.

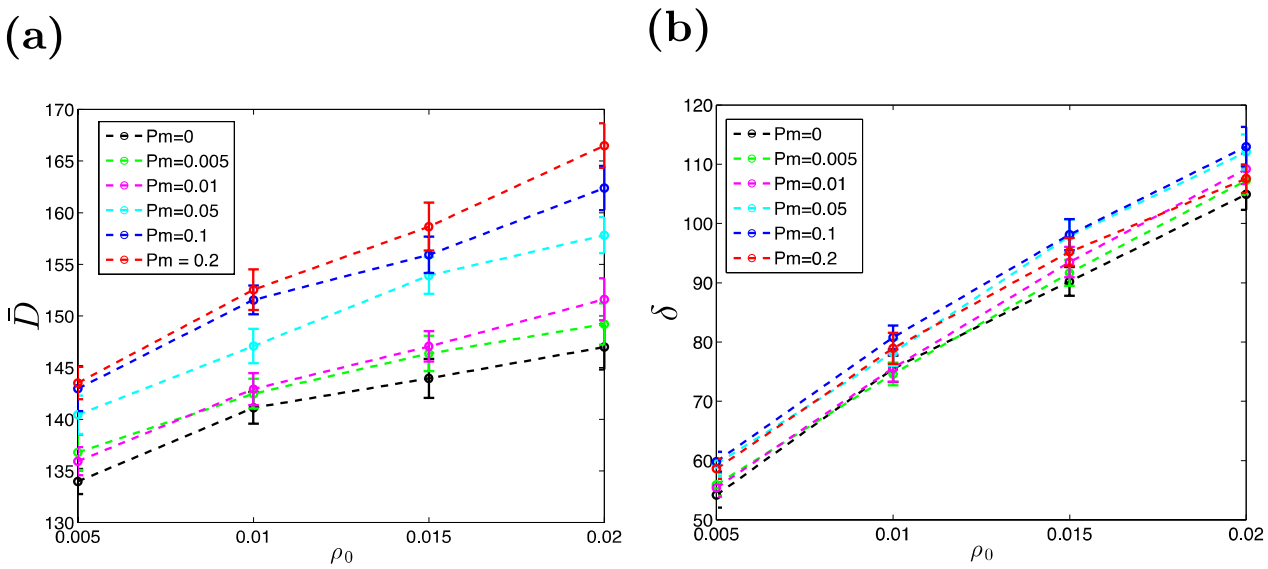

Figure S5 Microgel simulations with low values of Pm = {0, 0.005, 0.01, 0.05, 0.1, 0.2}. (a) standard deviation, δ, and (b) average, $\bar{D}$, of cluster size are depicted. The value of Pm for each curve is indicated in the legends. Other parameters are the same as for the simulations of Figure 9.

Table S1 Preparation of P(NIPAM-AA) microgels

NIPAM	AA	MBA	SDS	KPS
9.9 mmol	0.1 mmol	0.2 mmol	0.12 mmol	0.1 mmol
1.12g	6.86 μL	31 mg	35 mg	27 mg