On-chip high-throughput analysis of biomaterials viscoelastic properties using patterned suprhydrophobic surfaces

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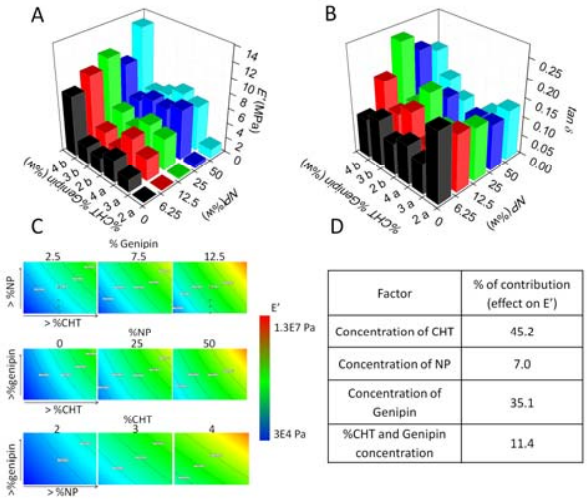
Statement of Purpose: Tissue-constituent cells are usually anchorage-dependent cells. Their viability is compromised when they are in a fluid suspension. The adhesion of these cells in the body occurs in solid elastic tissues. Mechanical properties of biomaterials were reported to have a role in modulating cells response, affecting their function and structure, as well as the direction of differentiation. It is well known that interactions occurring in the area of tissue engineering and regeneration are not easily predictable. As such, we previously developed arrays of wettable spots in polystyrene superhydrophobic surfaces in order to use these structures as high-throughput platforms for biomaterials development. We patterned biomaterials precursors in the wettable spots, keeping them confined in such regions by the wettability contrast caused by the superhydrophobic surroundings (1). By using the same type of chips developed for cells-biomaterials interactions studies, we aimed to adapt the system for the rapid study of miniaturized biomaterials viscoelastic properties. Most living tissues show a viscoelastic behavior, i.e., besides showing a particular stiffness, they have the ability to dissipate energy during cyclic stimulation. We used superhydrophobic chips to pattern miniaturized hydrogels, and adapted a mechanical dynamic analyzer (DMA) so on-chip viscoelastic properties of those materials could be assessed under physiological-like conditions.

Methods: For the proof-of-concept we performed a three-factor combinatorial study targeting bone tissue engineering applications. A system consisting of distinct combinations of polymeric matrix concentration, crosslinking degree and addition of biomineralizable bioglass nanoparticles (NP) was systematically studied. PVC stickers (Oracal® 614) were cut in a squared-shaped (2x2 mm^2). Arrays of 30 squared-shaped stickers separated by 2 mm were printed in polystyrene flaks. The polystyrene surfaces were then treated using a phase-separation method, as described elsewhere (2). Solutions of LMW chitosan 2% (w/v), 3% (w/v) and 4% (w/v) were prepared. These solutions were mixed with NP in amounts of 0%, 6.25%, 12.5%, 25% and 50% relatively to the chitosan total mass. Droplets of combinatorial solutions were dispensed in each individual spot of the chip and crosslinking reaction was left to occur for 3 h. The viscoelastic measurements of the scaffolds were performed using a TRITEC8000B DMA from Triton Technology (UK), equipped with the compressive mode, in PBS at 37ºC. A steel cylindrical probe was especially fabricated to perform non-destructive compression tests to the individual scaffolds in the chip. This piece was inserted in the fixed upper plate of the DMA equipment. In each essay, the individual scaffolds were vertically aligned with the cylindrical steel probe. Factorial analysis was performed to storage modulus (E') data obtained at 1 Hz was analyzed using Design-Expert7® software.

Results: An increase in the E' values with the increasing amount of mixed NP in the polymeric matrix was observed in all conditions. In general, an increase in E' values was also observed with increasing polymer concentration as well as with higher genipin concentrations. Loss factor values (Figure 1B) showed that the materials have, in general, a viscoelastic behavior.



Factor	% of contribution (effect on E')
Concentration of CHT	45.2
Concentration of NP	7.0
Concentration of Genipin	35.1
%CHT and Genipin concentration	11.4

Figure1. Average (A) E' and (B) tan δ values of the biomaterials measured on chip. (C) Surface response plots generated from the values measured o (A). (D) Percentage of contribution of each factor and combination of factors on biomaterials' storage modulus.

Surface response models were generated using the measured E' values (Figure 1C). The percentage of contribution of each factor is represented in Figure 1D. We observed that the major factor affecting E' in the system was chitosan concentration. The exposure of the chitosan/NP composite to different amounts of genipin was the second most influencing factor, followed by the combined effect of those two factors. The combined effect of chitosan concentration and genipin concentration was expected, as both factors are interdependent. NP amount was the less important effect.

Conclusions:

We performed a three-factor combinatorial study on the effects of composite hydrogels composition in biomaterials' viscoelastic properties. This was performed in a high-throughput manner, by adapting DMA testing to superhydrophobic patterned chips with biomaterials.

References: (1) Oliveira MB *et al*. Small. 2013;9:768-778. (2) Oliveira NM *et al*. Appl Phys Express. 2010;3:8.