High-throughput analysis of cells-3D biomaterials interactions using superhydrophobic patterned surfaces as chips

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Statement of Purpose:

Obtaining products with optimized characteristics is one of the main challenges in biomaterials development field. In tissue engineering this task may be carried out by combining biomaterials, cells and soluble factors in order to find out composition with the most promising results for specific applications. However, the unpredictability of such studies leads to the need of performing time- and resources-consuming tests. High-throughput methodologies have been proposed to master such complexity. However, such tests are performed using cells cultured into 2D biomaterials, although in native tissues cells lie in 3D milieus. We proposed the use of affordable and cytocompatible polystyrene superhydrophobic surfaces patterned with wettable regions as chips for the high-content study and image-based tracking of cells-3D biomaterials interactions. In such platforms each biomaterial is dispensed in the wettable spots and isolated from the rest of the chip by the surrounding highly-repellent surface. In a first approach, we used such chips to study cells-biomaterials interactions in cell-laden alginate-based hydrogels (1). However, to the best of our knowledge, high-throughput miniaturized systems had never been used before to study of one of the most commonly used structures in tissue engineering field: porous scaffolds. As such, we developed chips with the same concept, where biomaterials were processed based on a complexation and freeze-drying technique, prior to cell seeding. On such arrays we not only studied distinct cell types responses while in contact with biomaterials; we also characterized on-chip the biomaterials morphometrically and mechanically by non-destructive methods. All results were compared with samples produced by a conventional way, so the validation of the on-chip methodologies was accomplished (2).

Methods: Superhydrophobic surfaces were prepared as reported elsewhere (3). Hydrophilic regions were patterned on the surfaces by exposure to UV/Ozone irradiation using hollow masks. For the study using cell-laden hydrogels, alginate was mixed with L929 or MC3T3-E1 cells. On the chip, increasing amounts of collagen, chitosan or hyaluronic acid were added to the dispensed droplets of alginate/cells. The addition of gelatin to such mixtures was also studied. All materials were further crosslinked with $CaCl_2$. We studied cell viability and cell number on-chip by performing live/dead (calcein AM/propidium iodide) staining, and cell number (DAPI nuclei staining). The image-based cell quantification was performed using WCIF ImageJ. For the study of porous scaffolds, mixtures of alginate and chitosan were dispensed in the wettable spots, giving rise to a complexation reaction. Afterwards, the chips were freeze-dried. The obtained porous structures were characterized by dynamic mechanical analysis (storage modulus) and by microcomputed tomography (pore size and porosity). The biomaterials were then seeded with L929 or SaOs-2 cells. Image-based analysis was performed in the same way as the one to hydrogels.

Results: Superhydrophilic spots with controlled shape were successfully fabricated in the superhydrophobic surfaces. Regarding cell-laden hydrogels studies, after 24 hours of cell culture we observed that the composition of the hydrogels affected cell response. Mainly, pre-osteoblasts (MC3T3-E1) showed preference for chitosan and collagen-containing materials with lower amounts of alginate, while L929 cells preferred collagen and hyaluronic acid-containing materials, richer in alginate. The comparison of on-chip cell quantification by image and conventional tests (MTS and dsDNA quantification) were coherent, proving the suitability of image-based techniques. Regarding porous scaffolds studies, it was observed, in general, that the addition of alginate to chitosan led to increase in both porosity and stiffness. Both fibroblasts (L929) and osteoblast-like cells (SaOs-2) responded to the distinct characteristics of the biomaterials (Figure 1).

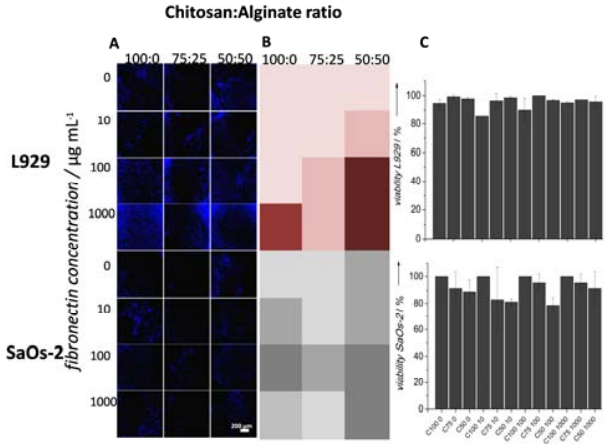

Figure 1. (A) Cell number, (B) respective intensity map and (C) viability of cells cultured on-chip in porous scaffolds.

Conclusions: Superhydrophobic patterned chips are adequate platforms for on-chip study of biomaterials. We believe they have a wide range of applications in distinct areas such as cell-materials interactions studies, gene therapy and high-throughput analysis of the effect of different types of irradiation and drugs in tumor cell death.

References: (1) Salgado CL *et al.* Integr Biol. 2012;4:318-327. (2) Oliveira MB *et al.* Small. 2013;9:768-778. (3) Oliveira NM *et al.* Appl Phys Express. 2011;3:8.