# 2D Microscale Engineering of Novel Protein based Nanoparticles for Cell Guidance

Witold I. Tatkiewicz,[1,2] Joaquin Seras-Franzoso,[2,3,4], Elena García-Fruitós,[2,3] Esther Vazquez,[2,3,4] Nora Ventosa,[1,2] Imma Ratera,[1,2] Antonio Villaverde,[2,3,4] and Jaume Veciana[1,2]

[1]Department of Molecular Nanoscience and Organic Materials, Institut de Ciencia de Materials de Barcelona (CSIC), Bellaterra, 08193 Barcelona, Spain,
[2]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Bellaterra, 08193 Barcelona, Spain,
[3]Institut de Biotecnologia i de Biomedicina (IBB), Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain,
[4]Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain,

wtatkiewicz@icmab.es, iratera@icmab.es, jveciana@icmab.es

**Abstract**

The term “inclusion bodies” (IBs) was coined to describe optically opaque moieties present in cell lumen. They have aspect of refractile particles of up to a few hundred nanometers and about 2 $\mu m^3$ of size when observed by optical microscopy and as electron-dense aggregates without defined organisation by transmission electron microscopy.[1]

The history of IBs turned when they were recognized as a prospective biomaterial with desirable properties. Being a product derived from biological synthesis, it is fully biocompatible and preserves the functionality of the embedded protein [2]. In a course of investigation it was revealed that IBs size, geometry, stiffness, wettability, z-potential, bio-adhesiveness, density/porosity etc. can be easily fine tuned by control over basic parameters of their production: harvesting time, host genetic background and production conditions (e.g. temperature, pH) In addition, their production and downstream processes are fully scalable, cost effective and methodologically simple.[3]

It is widely accepted, that cell´s responses, such as positioning, morphological changes, proliferation, mottility and apoptosis are the result of complex chemical, topographical and biological stimuli. Here we will show the application of IBs, as a functional biomaterial for engineering two dimensional substrates for cell guidance. We have cultivated fibroblast cells on supports patterned with IBs derived from green fluorescent protein (GFP) or human basic fibroblast growth factor (FGF). Two methodologies of pattern deposition were applied: microcontact printing (μCP) optimized for use with aqueous colloidal suspensions and a novel, template-free technique based on the coffee-drop effect due to a convective self-assembly (Figure 1).[4]

The first technique was applied in order to deposit IBs with high resolution geometrical patterns of various shapes and sizes. Then we have investigated how cells react to IBs geometrical distribution. Parameters such as orientation morphology and positioning were thoroughly investigated based on rich statistical data delivered by microscopy image treatment (Figure 2)The second technique has been recently developed in order to deposit complex and well-controlled two dimensional IB´s patterns with concentration gradients for the study of cell motility (Figure 3). Cell movement cultivated on such substrates was characterized and quantified based on confocal microscopy time-lapse acquisitions.[5,6]

In both cases a deep statistical data treatment was preformed to characterize macroscopic responses of cells when grown over nanoscale profiles made with IBs concluding that cell proliferation is not only dramatically stimulated but cell also preferentially adhere to IBs-rich areas, align, elongate and move according to such IBs geometrical cues.. These findings prove the potential of surface patterning with functional IBs as protein-based nanomaterials for tissue engineering and regenerative medicine among other promising biomedical applications.

**References**

[1] (a) Villaverde A., Carrio M.M., *Biotechnol. Lett*., **2003**, *25*, 1385 (b) E. García-Fruitós, E. Rodríguez-Carmona, C. Diez-Gil, R. Mª Ferraz, E. Vázquez, J. L. Corchero, M. Cano-Sarabia, I. Ratera, N. Ventosa, and J. Veciana, A. Villaverde *Adv. Mater*., **2009**, *21*, 4249 (c) Cano-Garrido, O.; Rodríguez-Carmona, E.; Díez-Gil, C.; Vázquez, E.; Elizondo, E.; Cubarsi, R.; Seras-Franzoso, J.; Corchero, J. L.; Rinas, U.; Ratera, I.; et al.. *Acta Biomater*. **2013**, 9, 6134.

[2] García-Fruitós E., Vazquez E., Díez-Gil C., Corchero J.L.; Seras-Franzoso J., Ratera I., Veciana J., Villaverde A., *Trends Biotechnol*., **2012**, *30*, 65

[3] (a) García-Fruitos E., Seras-Franzoso J., Vazquez E., Villaverde A., *Nanotechnology*, **2010**, 21, 205101 (b) Vazquez E., Corchero J. L., Burgueno J.F., Seras-Franzoso J., Kosoy A., Bosser R., Mendoza R., Martínez-Láinez J.M., Rinas U., Fernandez E., Ruiz-Avila L., García-Fruitós E., Villaverde A., *Adv. Mater*., **2012**, *24*, 1742 (c) E. Vazquez , M. Roldán , C. Diez-Gil , U. Unzueta, J. Domingo-Espín, J. Cedano, O. Conchillo, I. Ratera, J. Veciana , X. Daura, N. Ferrer-Miralles, A. Villaverde, *Nanomedicine*., **2010**, *5*, 2, 259

[4] (a) Han W., Lin Z., *Angew. Chem. Int. Ed*., **2012**, *51*, 1534 (b) Hanafusa T.,Mino Y., Watanabe S., Miyahara M.T., *Advanced Powder Technology* **2014**, *25*, 811

[5] (a) C. Díez-Gil, S. Krabbenborg, E. García-Fruitós, E. Vazquez, E. Rodríguez-Carmona, I. Ratera, N. Ventosa, J. Seras-Franzoso, O. Cano-Garrido, N. Ferrer-Miralles, A. Villaverde, J. Veciana, *Biomaterials*, **2010**, *31*, 5805 (b) J. Seras-Franzoso, C. Díez-Gil, E. Vazquez , E. García-Fruitós , R. Cubarsi, I. Ratera, J. Veciana, A. Villaverde, *Nanomedicine*, **2012,** *7(1)*:79-93

[6] W. I. Tatkiewicz, J. Seras-Franzoso, E García-Fruitós, E. Vazquez, N. Ventosa, K. Peebo, I. Ratera, A. Villaverde, J.Veciana, *ACSNano*, **2013,** *7(6)*, 4774

**Figures**

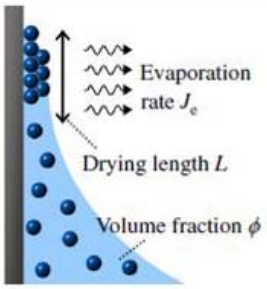


**Figure 1.** Schematic illustration of particle deposition. Particles are pinning to the substrate on the edge of meniscus, where the evaporation is more intense. Image adapted from reference [4b].

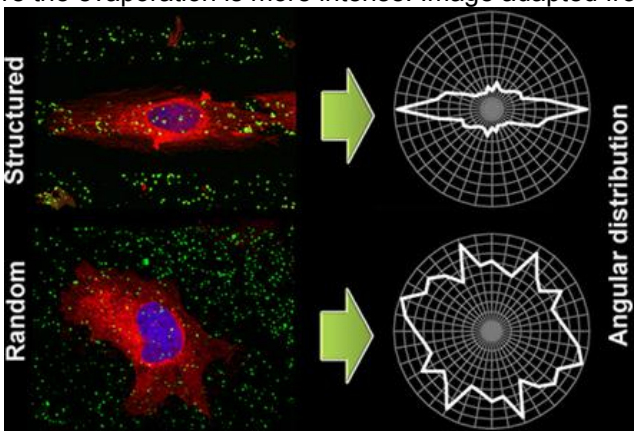


**Figure 2.** IBs striped (top) and random (bottom) pattern are compared. On the left; representative confocal microscopy images of cells cultivated on such patterns are presented. On the right; the overall orientation distribution of cells is presented. It is clearly seen, that cells are guided by the stripped pattern and they orient themselves along its geometry, whereas no predominant orientation of cells can be observed in the case of random pattern.

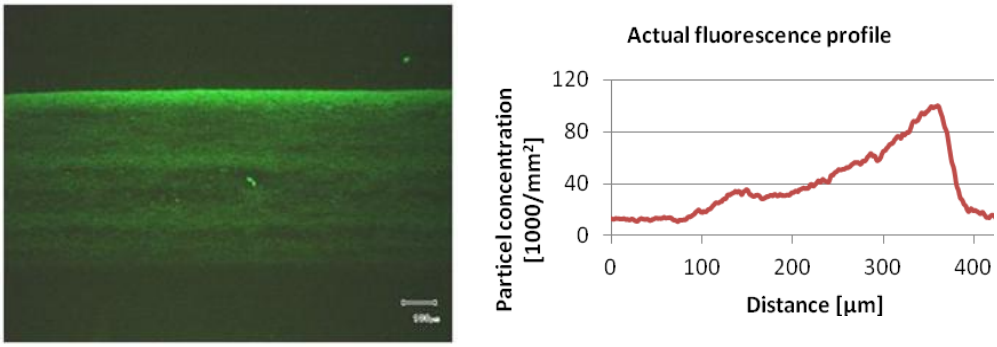


**Figure 3.** Example of GFP-derived IBs gradient pattern deposited by a controlled convective self-assembly technique. Left: fluorescence microscopy image, right: IBs concentration calculated based on fluorescence intensity.