Surface Cell Growth Engineering Assisted by a Novel Bacterial Nanomaterial and the Impact of Genetic Tailoring on Their Properties

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Bacterial inclusion bodies (IBs) are highly pure protein deposits in the size range of a few hundred nanometers produced by recombinant bacteria.[1] Although IBs were supposed to be undesirable side products on protein transcription processes it has been recently probed that those intriguing nanoparticulate proteic materials retain part of their original functionality and further more that it is possible to tailor its properties during biological production. The polypeptide chains that form IBs fold into an unusual amyloid-like molecular architecture compatible with their native structure, thus supporting the biological activities of the embedded polypeptides (eg fluorescence or enzymatic activity).[2] Therefore, a wide spectrum of uses as functional and biocompatible materials might arise upon convenient engineering.[3] Although theoretically feasible through adjusting genetic and production conditions, the biophysical features of these proteinaceus nanoparticles, such as activity and size, have been never engineered and very few is known about their physicochemical properties.

As IBs are biofunctional by nature, engineering of IBs might have wide applications in different nanomedical scenarios. In this study (Figure 1), we have characterized the relevant nanoscale properties of IBs as particulate materials using Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), Atomic Force Microscopy (AFM) and Confocal Microscopy (CM). We have demonstrated that these particles are mechanically stable and fully biocompatible, and that their size and biological activities can be tailored by appropriate genetic and process engineering.[4-6] Moreover, wettability and nanomechanical studies developed on IBs produced in different Escherichia coli genetic backgrounds depict distinguishable characteristics within the proteinaceous nanoparticles. (Figure 2 and 3).[5] As a proof of concept of the biomedical potential of IBs we have modified the topology of surfaces suitable for mammalian cell culture by adsorbing these particles, resulting in a dramatic stimulation of cell proliferation.[6] Furthermore, IB-grafted surfaces have been produced by using the microcontact printing (mCP), on which cell growth has been driven in desired patterns (Figure 1 g-i). Furthermore it was possible to observe how cultured mammalian cells respond differentially to inclusion body variants when used as particulate materials to engineer the nanoscale topography, proving that the actual range of referred mechanical properties is sensed and discriminated by biological systems.[5] The unique properties of this proteinaceous material including biocompatibility, manipulability and functionality make it especially appealing for regenerative medicine.

References

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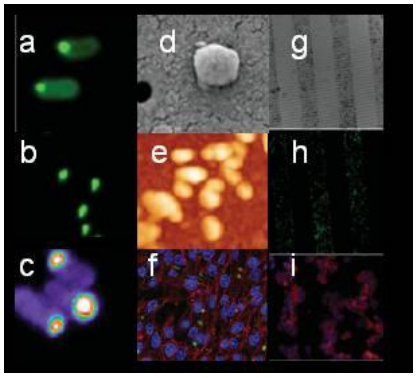

Figure 1. *IBs formed by the green fluorescence protein (GFP) when produced in bacteria (a), after purification (b), and under confocal analysis for florescence mapping (c). In (d) and (e), purified inclusion bodies observed by SEM and AFM respectively. In (f), BHK21 cells growing on polystyrene plates decorated with GFP IBs. In (g), a silica surface patterned with pure GFP IBs, that still being fluorescent (h), drive the growth of BHK 21 cells under the same lineal pattern (i).*

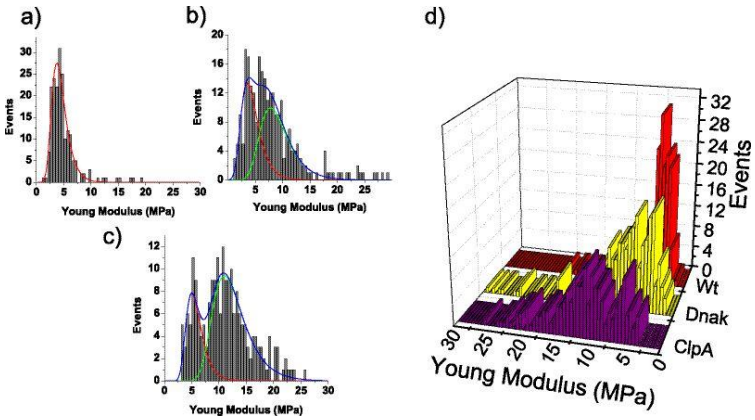

Figure 2. *Histogram representation of the number of events vs. Young modulus for IBs produced in bacterial mutants. a) Wt IBs showing only one peak at 3.73 MPa; b) DnaK- IBs show two overlapped Young modulus distributions which centered at 3.56 and 7.75 MPa; c) ClpA- IB show the presence of two different young modulus distributions, at 5.01 and 10.99 MPa; d) 3D representation of the later histograms.*

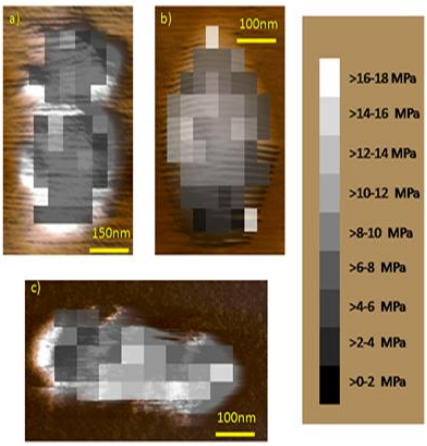

Figure 3. *2D software developed reconstruction of the elasticity maps of selected IBs from the three genetic backgrounds. a) wt IBs, b) DnaK- IBs, c) ClpA- IBs. Observations infer the existence of a homogeneously spread distribution of Young modulus values over the wt IBs. On the other hand, maps obtained for DnaK- and ClpA- IBs indicate the existence of two elasticity populations, with the harder areas segregated and localised on the centre of the DnaK- IBs and on the right side of ClpA- particles.*