Heterogeneous polymer surfaces with organized collagen layers influence preosteoblasts behavior

Emilienne Zuyderhoff, Christine Dupont-Gillain.
Institute of Condensed Matter and Nanosciences, Université catholique de Louvain, Louvain-la-Neuve, Belgium

Introduction: The extracellular matrix (ECM), which provides physical and (bio)chemical cues for the regulation of cell behavior, presents a complex structure on the micro- and nanoscales. The creation of environments mimicking the ECM is a challenge of importance in biomaterials science and tissue engineering. Type I collagen, a self-assembling protein, is one of the main components of ECM, and provides adhesion signals to cells. The supramolecular organization of collagen at interfaces is known to depend of the physico-chemical properties of the substrate, including its wettability. The aim of the present work is to create a variety of biointerfaces with different supramolecular organizations of type I collagen, obtained by collagen adsorption on chemically heterogeneous surfaces. Then, the behavior of preosteoblasts cultured on the designed biointerfaces is investigated.

Methods: *Preparation of organized collagen layers:* Polymer demixing upon spin-coating was used to create surfaces containing hydrophobic inclusions in a more hydrophilic matrix or conversely, with a view to further create heterogeneity in the supramolecular organization of adsorbed collagen. The immiscible polymers used for demixing were polystyrene (PS) and poly(methyl methacrylate) (PMMA). Several polymer concentration ratios and solvents were tested in order to obtain a range of substrates showing different nanoheterogeneities. Collagen adsorption was then performed on homogeneous and heterogeneous substrates by immersion for 2 h in 30 µg/ml collagen solution in phosphate buffer. The topography of the surfaces obtained before and after collagen adsorption was assessed by atomic force microscopy (AFM), while their surface chemical composition was determined using X-ray photoelectron spectroscopy.

Cell culture: A murine osteoprogenitor cell line, MC3T3-E1, was cultured on the created protein layers. Cell morphology and density were investigated after 4h and 6 days in serum-containing medium, using fluorescence microscopy (actin, nucleus and vinculin labeling).

Results: After spin-coating of several PS/PMMA blends, four interesting substrates were identified: substrates with PMMA inclusions in a PS matrix, and conversely, substrates with PS inclusions in a PMMA matrix, the inclusions being either under the form of pits or islands. The obtained surface morphologies could be linked to the respective solubility of the polymers in the solvents used.

The organisation of collagen layers was different depending on the nature of the polymer substrate (Figure 1): the layer was quite smooth on pure PMMA, while large assemblies were found on pure PS. Collagen adsorption on heterogeneous substrates led to surfaces presenting a heterogeneous distribution of collagen. The more the substrate contained PS, the more the collagen layer contained fibrils. Moreover, when PS domains size was lower than ~600 nm, assemblies larger than the ones observed on pure PS were formed.

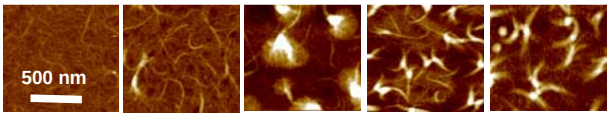

Figure 1. AFM images showing collagen organization on PS/PMMA blends, in function of PS surface fraction (from left to right: 0, 15, 21, 64 and 100 % PS)

MC3T3-E1 cells adhered on all collagen-coated surfaces. Cell density remarkably increased on heterogeneous surfaces compared to homogeneous ones, especially when very large collagen fibrils were present. Besides, the observed cell morphologies were also different depending on the biointerface architecture. Quite small and round cells were found on the smooth collagen layer formed on PMMA, while cells were bigger and more elongated on the collagen fibrils found on PS. Intermediate cell morphologies were observed on the heterogeneous PS/PMMA surfaces after collagen adsorption.
After 6 days, remodeling of the collagen layer probably occurs, and while a strong effect of substrate heterogeneity is still observed, the same effect is also found on the naked substrates, in absence of collagen.

Conclusions: The present work demonstrates that chemical and topographical nanoheterogeneities, obtained by polymer demixing, interfere with collagen adsorption. The protein layer organization (smooth layers vs supramolecular assemblies; size of assemblies) is indeed altered by the presence of domains of different chemical nature (PMMA vs PS) at the surface of the substrate used for adsorption. Culture of preosteoblasts highlights major differences between cell behaviors observed on the different biointerfaces obtained by collagen adsorption on pure PMMA, PS/PMMA heterogeneous surfaces, and pure PS. A better control of cell-material interaction may thus be obtained through tailoring of ECM protein layers.