Utilization of Mechanical Properties´ Imaging for Detection of Au-nanospheres Used as Biomarkers

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Introduction and Materials

The development of bioactive artificial material mimicking the natural signals is actually advanced approach for guided tissue regeneration and/or tissue engineering. The patterned surfaces carrying biomimetic structure, e.g. fibronectine, are required to facilitate functionalized cell adhesion, migration, growth or differentiation.

Specific distribution of cell-adhesion structure over the biomaterial surface is highly desirable, but problematically visualized. Biomaterial surface (functionalized PLA-b-PEO copolymers with controlled polylactide (PLA) and polyethylenoxide (PEO) block) was patterned to cell adhesion structure by biotin labeled fibronective derivate peptides (RGDS functional group) with different spatial distribution (random or clusters). Streptavidin labeled Au – spherical nanoparticles (40 nm) - NS were used as marker for biotin labeled peptides due to strong interaction (streptavidin – biotin), low mass of Au-NS and a high contrast in mechanical properties between PLA-b-PEO polymer and Au-NS. Unfortunately, Au-NS were not detectable neither by SEM and TEM (damage/heating of the film, movement of Au-NS) or simple topological image of the surface by AFM (roughness of the surface is comparable to Au-NS).

Mapping of mechanical behavior was used for visualization of patterned surface due high contrast between hard Au-NS vs. very soft PLA-b-PEO matrix, namely employing AFM in acoustic mode (AFAM), phase shift image and force spectroscopy.

Results and Discussion

Atomic Force Acoustic Microscopy (AFAM)

AFAM [1] allows the measurement of local elastic properties (stiffness and density of surface and near-sub-surface matter). It is based on the forced out-of-plane vibration of the sample, where the movement of the surface is detected by AFM tip. This method allows detection of Au-NS, however the Au-NS was pushed down during measurement and fluctuation of elastic properties of PLA-b-PEO matrix has similar intensity signal as Au-NS (data not shown in abstract).

Phase shift image

This method [2] is based on mapping of changes of phase shift between oscillations of the free and fixed cantilever ends. It allows a detection of all probe-surface interaction (capillary forces-water, stiffness, van der Waals forces, adhesion forces in general). In this semi-contact AFM mode, the Au-NS particles were detected and the different surface patterns were recognized (random vs. cluster distribution - see Fig. 1) and this method was robust for tip selection (spring constant 1.7 – 14 N/m). The PLA-b-PEO polymer embodies significantly lower difference in probe-surface interaction in comparison with Au-NS.

Force spectroscopy

Force spectroscopy is used for proof of “harder” properties of Au-NS particles with diameter ca 50 nm visualized by phase shift image (Figs. 1, 2 – shown as blue areas), e.g. as verification of Au-NS. It is based on a relation of cantilever deflection as a function of piezo-displacement along the z-axis [3] and it allows detection of stiffness of the material and capillary forces superlatively. It was proved the different stiffness of materials soft PLA-b-PEO (-14.4±0.5) vs. hard Au-NS (-19.1±0.5), see Fig. 2. The disadvantages were mainly i) worse spatial resolution of the image compared with phase shift image, ii) necessity of usage of contact cantilevers for phase shift image and force spectroscopy and iii) time consuming experiment.

Conclusion

There was set up a method for detection the pattern of cell adhesion structure on biomaterial surface using Au-NS as marker in very soft polymeric material. Au-NS were detected by mapping of mechanical behavior of surface and random and cluster patterns were visualized on the surface and phase shift image resulted in adequate method for detection of Au-NS on this type of material.

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References
[1] U. Rabe, S. Amelio, E. Kester, V. Scherer, S. Hirsekorn, W. Arnold, Ultrasonics **38** (2000) 430.
[2] Y. Martin, C.C. Williams, H.K. Wickramasinghe, Journal of Applied Physics **61** (1987) 4723.
[3] B. Capella, P. Baschieri, C. Frediani, P. Miccoli, C. Ascoli, Ieee Engineering in Medicine and Biology Magazine **16** (1997) 58.

Figures

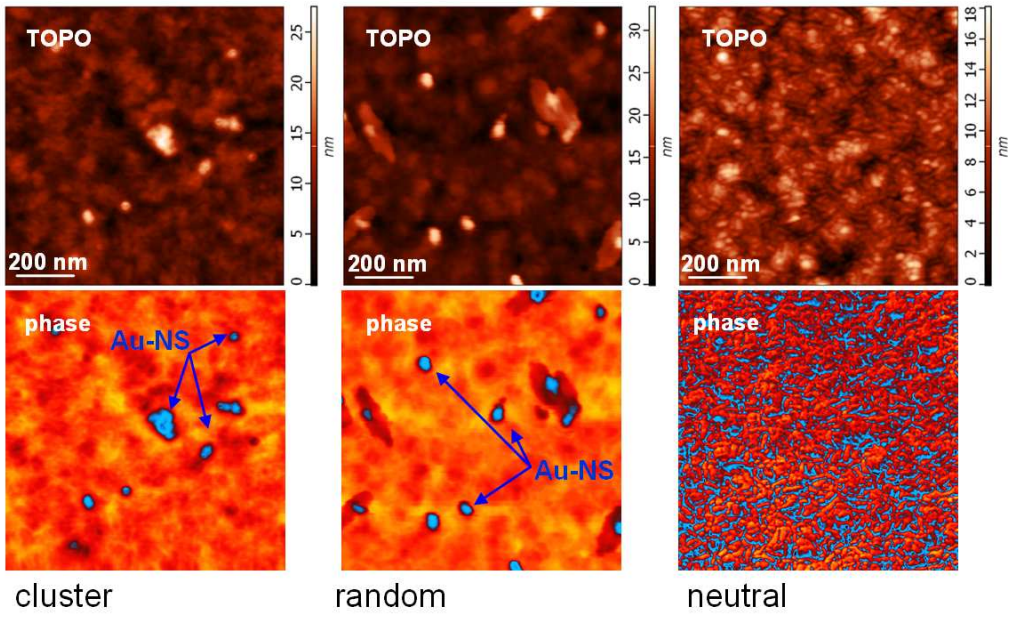

Fig. 1 AFM images of topology (TOPO) and phase shift images (phase) of Au-nanospheres on material with cluster and random distribution, neutral PLA-b-PEO surface without peptides is added for comparison.

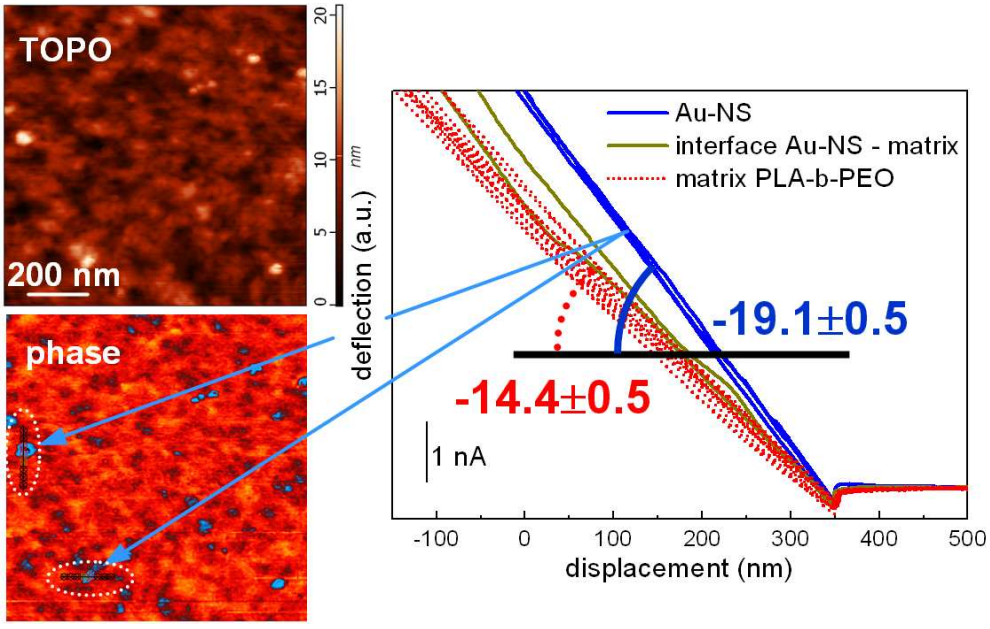

Fig. 2 Force spectroscopy and topological (TOPO) and phase shift image (phase) of Au-NS on PLA-b-PEO surface. The verification of Au-NS is based on different stiffness of Au-NS and polymeric matrix.