

Improved cell adhesion to plasma treated collagen surfaces

Introduction

Cell adhesion to biomaterials and implant surfaces is essentially influenced by their surface properties.¹ Not only the chemical composition, but also surface energy and hydrophilicity affect cell-material interactions and fundamental biological processes including cellular morphology, proliferation and differentiation of cells.² Manipulating surface properties is therefore important for various biomedical applications.

Collagen and collagen-based biomaterials are widely used in the medical field due to the high biocompatibility, low immunogenicity and bioresorbability. However influencing surface quality is usually associated with the use of solvents and the loss of biocompatibility. Surface functionalisation with plasma techniques is a possible method to manipulate physical and chemical properties of collagen without changing basic material properties.

Methods

In this study, collagen coatings and foils were functionalized with atmospheric plasma and gaseous fluorine, respectively, to investigate effects of a modified surface chemistry on cell adhesion. Plasma activation was performed as a dielectric-barrier discharge (DBD) in the presence of the process gases air, nitrogen or argon.

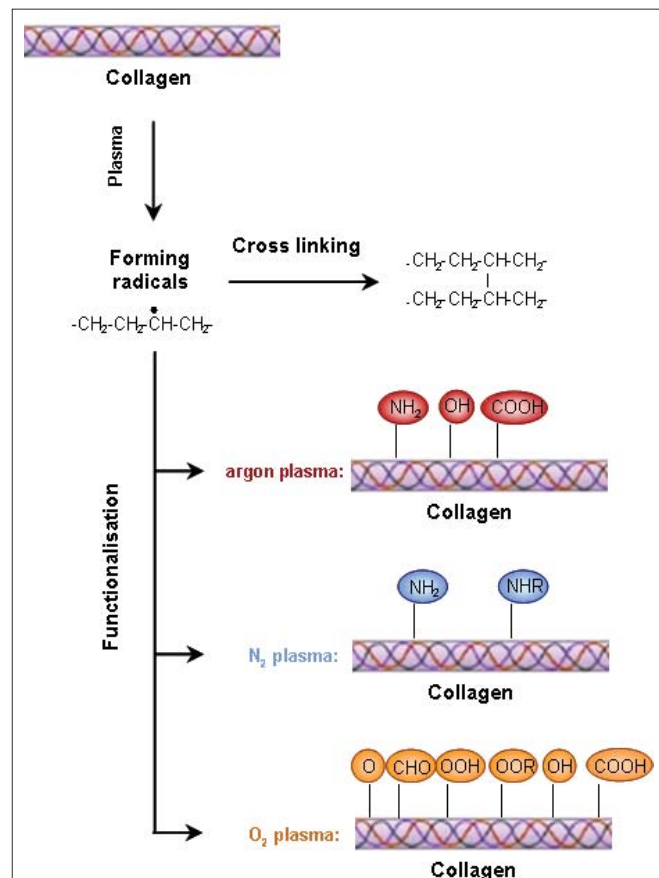


Fig. 1: Schematic illustration of the surface reactions depending on the plasma composition.³

The collagen surface was analyzed with contact angle measurements, X-ray photoelectron spectroscopy (XPS), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). Cellular response to manipulated collagen was investigated using the murine fibroblast cell line L929.

Results

Cell cultivation on functionalized collagen surfaces revealed two effects. Oxo-fluorination and plasma treatment with argon or nitrogen resulted in an increase in cellular adhesion and elevated cell density after three days of cultivation. Spreading area of the cells was remarkably increased especially for fluorinated collagen surfaces.

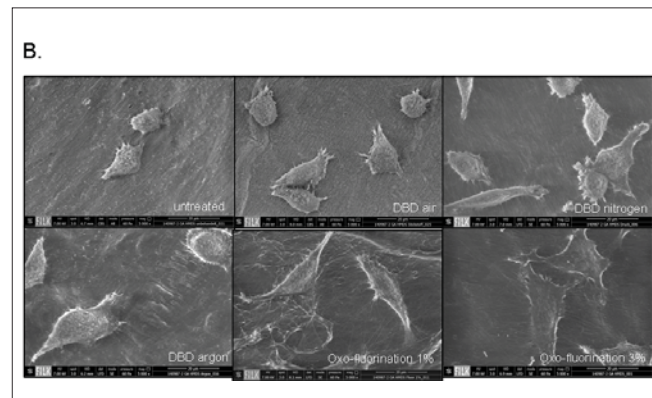
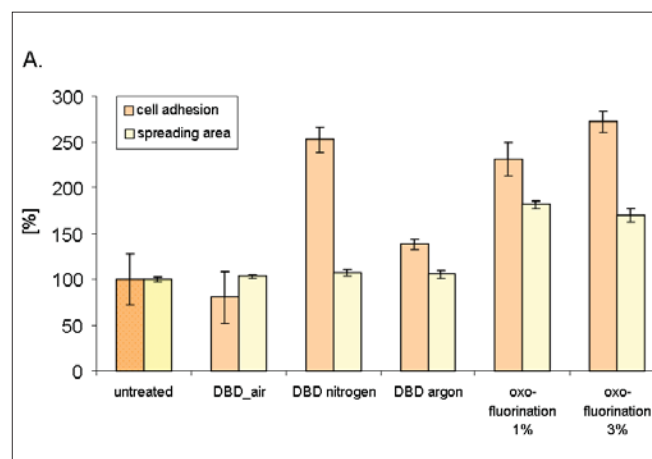


Fig. 2: A.) Cell adhesion and spreading area of mouse fibroblasts on functionalized collagen surfaces. B.) Electron microscope image of mouse fibroblasts two hours after seeding.

Contact angle measurements revealed significant improvement of surface wettability. Furthermore the polar part increased after functionalisation with atmospheric pressure plasma. Oxo-fluorination resulted in a weak decrease of the contact angle of water and the surface energy.

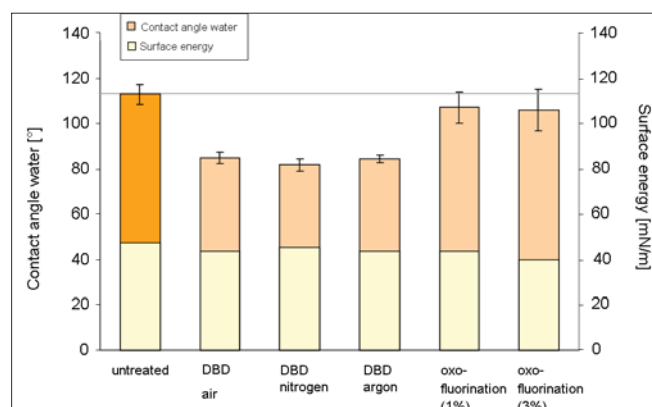


Fig. 3: Contact angle water and surface energy of functionalized collagen films.

XPS analyses indicate changes in elemental composition of treated collagen. Oxygen and nitrogen content was increased upon treatment. Furthermore, fluor atoms were found in the surface of oxo-fluorinated samples.

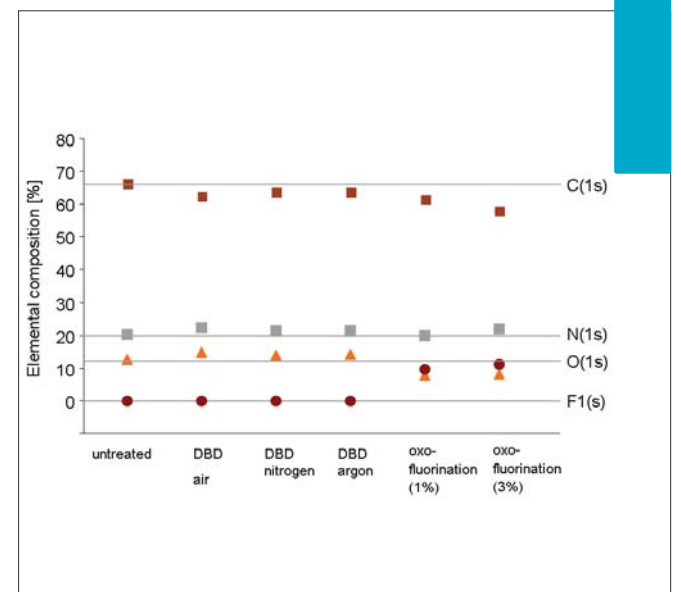


Fig. 4: Elemental composition of collagen surface after functionalisation.

To evaluate the effects of the plasma treatment on the thermal stability of collagen, DSC analysis were performed. DSC curves showed no changes in denaturation temperature, suggesting that the collagen was not denatured or crosslinked during functionalisation.

Conclusion

Interestingly, increased cellular adhesion to the modified surfaces was not detectable after the treatments with air-containing plasma indicating that improved cell adhesion can not be explained with hydrophilic surface properties. Furthermore, influences of the topography of the collagen surface on cell adhesion can also be excluded, because no differences could be observed with SEM.

So far the reason for the improved cellular adhesion is unknown and new surface sensitive methods are required to explain these variations.

By now, the results demonstrate that it is possible to influence cell adhesion to collagen in a biocompatible way without the use of solvents. Plasma techniques could thereby improve the properties of collagen and increase its applicability.

References

- Ventre M, Causa F, Netti PA (2012). Determinants of cell-material cross-talk at the interface: towards engineering of cell instructive materials. *J R Soc Interface* 9(74): 2017-2032.
- Liu X, Lim JY, Donahue HJ, Dhurjati R, Mastro AM, Vogler EA (2007). Influence of substratum surface chemistry/energy and topography on the human fetal osteoblastic cell line hFOB 1.19: Phenotypic and genotypic responses observed in vitro. *Biomaterials* 28(31): 4535-4550.
- Rieß K (2001). Plasmamodifizierung von Polyethylen. Dissertation, Martin-Luther-Universität Halle-Wittenberg.