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INFLUENCE OF PLASMA FUNCTIONALIZATION ON CELL ADHESION TO COLLAGEN

INTRODUCTION

Collagen is the main structural protein in various connective tissues in animals. It can form long fibrillar aggregates which are responsible for stability, form, tensile strength and flexibility of tissues. Due to the high biocompatibility, collagen is a well accepted and widely used biomaterial (hemostyptic sponges, wound dressings, scaffolds for tissue engineering and drug delivery systems). Beside the biocompatibility collagen is biodegradable, only slightly immunogenic, highly versatile and a suitable cell carrier [1]. However, controlling cell adhesion to this biomaterial is limited [2, 3].

MOTIVATION

The aim of the research is to modify the surface properties of collagen devices by plasma treatment and oxo-fluorination, respectively, in such a way that cell adhesion can be adapted depending on the application. For example, increased cell attachment should be achieved for implants or tissue engineering constructs, while a decrease in cellular adhesion is preferred for intraocular lenses or artificial heart valves.

EXPERIMENTAL

The modified collagen films were characterized by physical methods and in cell culture.

Thus, the whole process can be summarized as follows:

1. MATERIALS

- Unsterile collagen films made of unsoluble collagen
- Flexible, thickness of 20 µm, not cross-linked

2. MODIFICATION OF COLLAGEN FILMS

2.1 PLASMA FUNCTIONALIZATION

- Plasma is an electrically neutral medium of charged and neutral particles
- Surface reaction, polymerization and etching [4 - 6]
- Dielectric-barrier discharge (DBD) (Fig. 1):

  a) Oxygen and oxygenated plasma

  b) Nitrogenous plasma

  c) Argon plasma

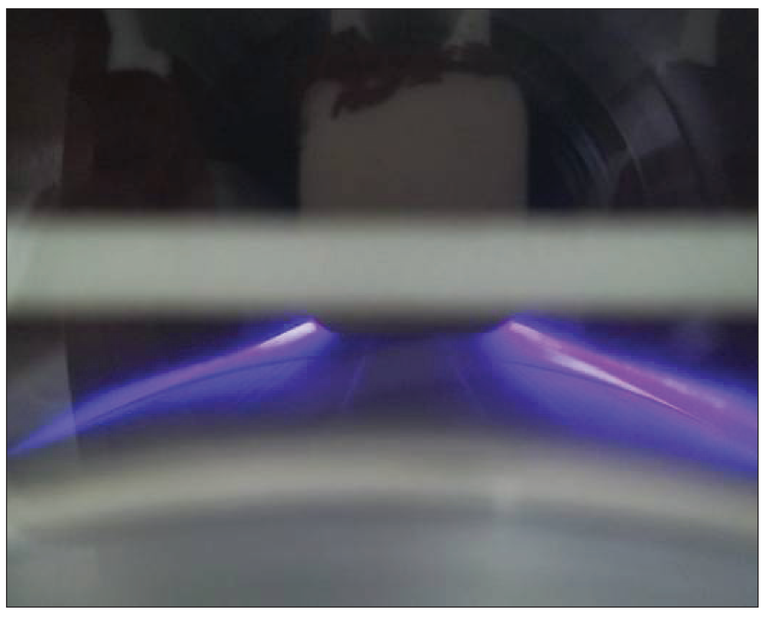

Figure 1: Dielectric-barrier discharge (DBD)

2.2 MODIFICATION BY OXO-FLUORINATION (Fig. 2)

- Fluorine/nitrogen mixture (ratio 10:90) in presence of air
- Hydrogen substituted for high reactively fluorine
- Irreversible process
- The presence of air additionally generates oxygen containing groups on the surface [6]

Figure 2: Pilot plant for oxo-fluorination

3. STERILIZATION BY GAMMA-RADIATION

4. CELL CULTIVATION

- Seeding fibroblasts
- Cell adhesion and spreading (Fig. 3)

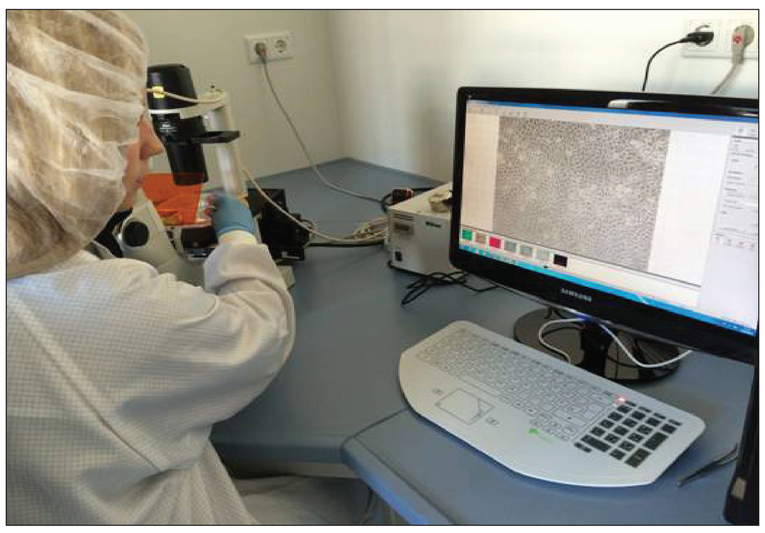

Figure 3: Investigation of fibroblasts

RESULTS AND DISCUSSION

Changes of the collagen surface after plasma activation were detected by contact angle measurements as presented in Fig. 4.

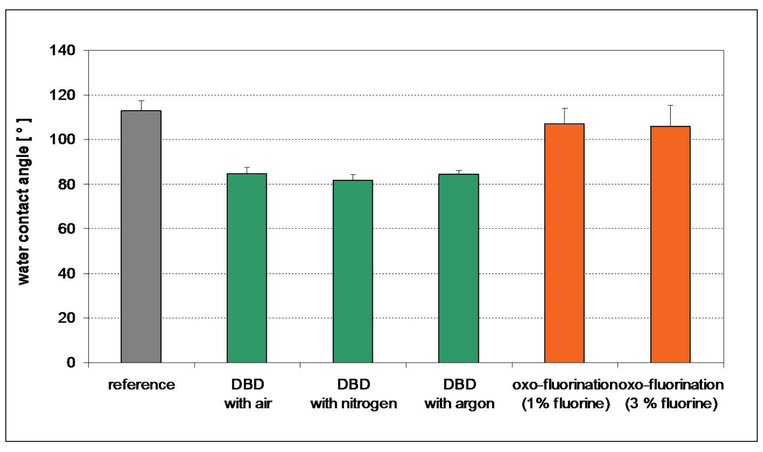

Figure 4: Water contact angles of functionalized collagen films

Fig. 5 shows the cell spreading of fibroblasts two hours after seeding on the collagen surface.

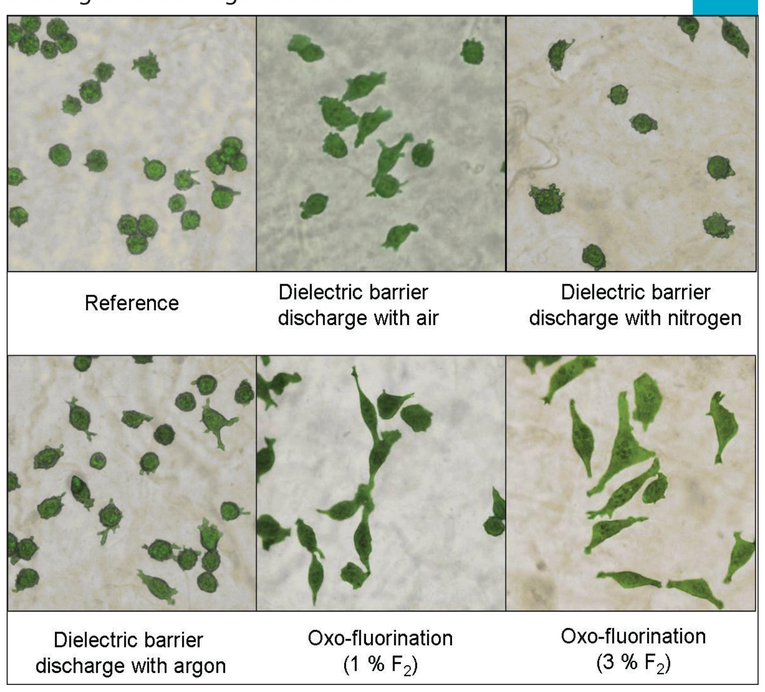

Figure 5: Cell spreading after 2 hours of incubation under standard culturing conditions

Plasma treatment with the process gases air or nitrogen had no effect on the adhesion of fibroblasts to collagen. However, plasma treatment with argon and oxo-fluorination, respectively, increased the number of adhering cells significantly (data not shown). Interestingly, the improved cell adhesion after these treatments could not be explained with changes of the hydrophilic properties. Furthermore, no influences on the topography of the collagen surface could be detected. Therefore, the reason for the improved cell adhesion is not known and new surface sensitive methods are required to explain these variations.

CONCLUSIONS

Collagen is a widely used biomaterial and is applied as tissue engineering scaffold *in vivo* and *in vitro*. However, controlling cell adhesion to this biomaterial is limited. Therefore, in this study collagen films were functionalized by atmospheric pressure plasma and oxo-fluorination to investigate effects of a modified surface chemistry on cell adhesion.

The results indicate that it is possible to influence cell adhesion to collagen without the use of solvents. The biocompatibility of the collagen remains in spite of the treatment. Furthermore, sterilization can be achieved by application of plasma [7]. Plasma techniques as well as oxo-fluorination can improve the properties of collagen and thereby increase its applicability.

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