Factors responsible for biomaterials modification in the electron-beam plasma

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Abstract. The modification of some biomaterials by the Electron-Beam Plasma was studied experimentally. The powder of the fibrin-monomer (the natural protein which is contained in the blood of mammals) was treated in the plasma generated by injecting the continuous electron beam in gaseous or vapor media. The fibrin-monomer was found to change its physical-chemical and biological properties due to the treatment. In particular, being modified the fibrin-monomer reduced the human platelet aggregation degree \textit{in vitro} to $\approx 33 – 35 \%$, whereas the untreated compound did not inhibit the aggregation. Both experiments and computer simulation were carried out to separate plasmachemical effects from other factors acting on the sample during the treatment ($\beta$-irradiation, UV- and X-ray radiation, heating). The study showed the fibrin-monomer to acquire the anti-aggregation activity due to combined action of all factors mentioned above but the plasmachemical processes are predominant.

1. Introduction

The Electron-Beam Plasma (EBP) is generated by injecting an electron beam (EB) into a gaseous medium. The EBP composition is complex: generally it contains molecules, atoms, radicals and ions in stable and excited states, plasma electrons and injected beam electrons as well. At moderate pressures ($P_{\text{m}} < 10^2$ Torr) the EBP is usually strongly non-equilibrium. It means that the function of the electron energy distribution in the EBP is non-Maxwellian and heavy plasma particles mentioned above are produced in super-equilibrium concentrations, i.e. very high densities of ionized and excited particles can be obtained. As a result, the EBP appears to be chemically active even at low temperature.

The EBP was proved to effectively modify properties of materials, complicated organic and polymer molecules being the most modifiable even at low temperature [1]. The following factors influence the material placed into the plasma cloud:

- chemically active heavy particles of the EBP (excited molecules and atoms, ions, radicals);
- the fast electrons of the partially degraded EB that bombard the sample; the secondary electrons of moderate energy produced in the EBP can also act on the powder;
- the EBP-radiation, especially UV one and X-ray (bremsstrahlung);
- possible heating by direct electron bombardment and due to the heat transfer between the plasma cloud and sample.

Each of these factors is able to cause transformations of macro-molecules, but the integral effect of the macro-molecule modification is likely to be due to their joint action, i.e. the synergism is expected to take place. As a result, being modified by the EBP, the bio-materials can exhibit unique properties.

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Fibrin-monomer (the natural protein which is contained in the blood of mammalians) was used as the testing bio-material to prove the efficiency of the of bio-macromolecules modification by the EBP.

2. The Electron-Beam Plasmachemical reactor and the treatment procedure

Figure 1 illustrates a typical procedure of the EBP generation. The continuous EB generated by the electron-beam gun located in the high vacuum chamber is injected into the reaction chamber filled with the molecular gas through the injection window (IW). Due to the interaction of the EB with the gas the plasma cloud is generated. A small portion of the EB power transforms into the radiation, as a result the optical radiation (including UV) and X-ray one are produced.

![Figure 1. The Principle of the EBP generation and the powder treatment procedure; the comments are given in the text.](image)

The reaction chamber is preliminary evacuated to pressure \( \approx 10^{-1} \) Torr and then filled with the plasma generating gas through the feeder. The pressure \( P_m \) varied within the range 1 – 100 Torr depending on the gas composition and treatment conditions required. The noble gases (He) and water vapor were used to generate the EBP. A specially designed double-stage gas-dynamic IW is used to transport the EB of energy \( E_b = 20 – 40 \text{ keV} \) from high vacuum \( \approx 10^{-5} \) Torr (that is required for the operation of the electron gun) into the reaction chamber filled with the gas.

The glass container (Petri cap) with the powder to be treated is inserted into the plasma cloud. The powder was additionally grinded by means of the laboratory mill “Puluerisette-23” (producer – company “Fritsch”) and the mono-layer of the substance was spread over the container bottom through the sieve. Average sizes of the powder particles were within the range 20 – 40 \( \mu \text{m} \). The temperature of the container bottom (and respectively of the powder) was monitored by the sensor.

Not only does the EB create the plasma cloud in passing through the gas, but it is able to heat the container and, under certain conditions, the plasma-generating medium. The miniature thermo-sensor is inserted into the container to monitor the material temperature during the treatment.

3. The calculation of the \( \beta \)-radiation doses accumulated by the bio-material powder treated in the EBP: the physical model and method of calculation

The fast electrons injected into the reaction chamber pass through the gas layer and then bombard the powder to be treated. On propagating in gas the originally thin beam is scattered due to the elastic collisions and the energy of electrons gradually diminishes due to the electron deceleration in inelastic collisions. Then, the scattered and decelerated beam reaches the powder layer and penetrates into it.
Thus, two problems should be considered separately: the beam propagation in gas and in solid medium, the approach to the modeling of the electron motion being the same for both problems.

The cross-sections of the elastic electron collisions with any elements of the periodic table are calculated by means of the Thomas-Fermi model within the range of the electron energies $5 \text{ eV} < E < 30 \text{ keV}$ [1], the calculation accuracy being about 20%. Following [1], to simulate the electron trajectories any media (gaseous, liquid or solid) are supposed to be the superposition of independent atoms.

The energy losses are calculated in terms of the Bethe formula:

$$- \frac{d\varepsilon}{dx} = 2 \cdot \pi \cdot r_0^2 \cdot \frac{(\varepsilon + 1)^2}{(\varepsilon + 2) \cdot \varepsilon} \cdot \sum n_i \cdot Z_i \cdot \left( \ln \left( \frac{m \cdot c^2}{E} \cdot \frac{\varepsilon^2 \cdot (\varepsilon + 2)}{2 \cdot I_i^2} \right) + f(\varepsilon) \right),$$  

(1)

where

$$f(\varepsilon) = \frac{1}{(\varepsilon + 1)^2} + \frac{1}{8} \cdot \frac{\varepsilon^2}{(\varepsilon + 1)^2} - \frac{2 \varepsilon + 1}{(\varepsilon + 1)^2} \cdot \ln 2,$$  

(2)

$\varepsilon = \frac{E}{m \cdot c^2}$ - relative electron energy; $r_0$ - classical radius of the electron; $n_i$ - concentration of medium atoms of the $i$-th kind, cm$^{-3}$; $Z_i$ - atomic number. The data regarding average values of the ionization potentials $I_i$ of atoms, which are used in our consideration, are given in [3].

The Monte Carlo method was used to simulate the EB propagation both in gaseous and solid media. The free path of the electrons $S$ is defined by the relationship (3) [6]

$$\int_0^S \sum n_i \cdot \sigma_i \cdot ds = -\ln(\xi),$$  

(3)

where $n_i$ - the concentration of atoms in medium in which the EB propagates, $\sigma_i$ - the collision cross-section for atom of $i$-th kind, $\xi$ - a random variable uniformly distributed over the segment [1].

The integral in (3) is calculated over the electron trajectory. The definition (3) of free path is applicable for electrons propagating in both homogeneous and inhomogeneous media and for electrons crossing the boundary between two media. The probability of the electron collision with the atom of $i$-th kind is proportional to $\sigma_i \cdot n_i$.

The angle $\theta$ of the electron scattering can be found using the data of the tables [1] which describe the dependence of the differential cross-section of the elastic collisions of electrons as a function of the angle value. The Rutherford formula can be used as well:

$$\cos(\theta) = 1 + \frac{2 \cdot \eta \cdot (\xi - 1)}{\eta + \xi},$$  

(4)

where $\xi$ is a random variable uniformly distributed over the segment [1], $\eta$ is the screening parameter that can be written as [2]:

$$\eta = 1,7 \times 10^{-5} \frac{Z^{2/5} \left(1 - \frac{\beta^2}{\beta^2} \cdot \eta_e \right)}{\beta},$$  

(5)

$$\eta_e = 1 + 4 \cdot \frac{Z}{137} \cdot \chi_0 \cdot \left( \frac{1 - \beta^2}{\beta} \cdot \ln \chi_0 + \frac{0,231}{\beta} + 1,448 \cdot \beta \right),$$  

(6)

$$\chi_0 = 0,00825 \cdot \frac{\sqrt{1 - \beta^2}}{\beta} \cdot Z^{1/3},$$

$$\beta = \frac{E}{m \cdot c^2}.$$ 


The procedure of numerical simulation of the electron propagation in dense medium includes:

- calculation of the electron free path length,
- calculation of the electron energy loss along this path,
- calculation of the scattering angle,
- assignment of the new position of the electron in 3D-space,
- assignment of the vector of the new direction of the electron motion.

To realize the procedure mentioned above special software was developed. The software is adjusted to real experimental unit (see section 2). To predict the β-radiation doses distribution over the sample volume exactly the following calculations are carried out: the EB absorption in the injection window, the EB scattering and absorption in the gas filling the reaction chamber, back-scattering of the electrons from the sample surface, propagation and absorption of the electrons in the sample bulk. Calculations can be performed not only for beams with fixed axis but for scanning beams. Various scanning techniques were used in experiments (sinusoidal or saw-tooth scanning in x- and y-directions, ring scanning) and all of them can be simulated. The software is able to calculate the dose accumulated by any elementary volume of the sample bulk to obtain and visualize 3D-pattern of the β-radiation absorption. The database of the software contains information sufficient for modeling the wide variety of the experimental conditions (plasma-generating gas composition and pressure, material of the sample and its density, peculiarities of the injection window and reaction chamber design).

The algorithm was tested by comparing with well-known results regarding the penetration of the electrons through thin foils within the electron energy range 100 – 300 keV [4], the back-scattering of the electrons in air within the electron energy range 1 – 25 keV [5]. In addition, the simulation results were compared with the data obtained in our experiments in which the wall temperature of the small-size reaction chamber filled with the EBP was measured at various points [6]. The comparison showed the results of simulations to be in a good agreement with the data of the articles mentioned above; the discrepancies do not exceed 1 – 3 %.

4. The calculation of the β-radiation doses for real conditions of the biomaterials treatment

The calculations were carried out for the following conditions:

- The initial energy of the electron beam – 25 keV.
- The plasma-generating gases – helium and water vapor.
- The ranges of the gas pressure variation – 10 – 50 Torr (for helium) and 1 – 15 Torr (for water vapor).
- The distance between the injection window and sample surface – 250 mm.
- The design and characteristics of the injection window are described in [6].

The EB was deflected in x- and y-directions by means of the electromagnetic scanning system. The coils of the scanning system were fed by sinusoidal voltages with frequencies 50 and 450 Hz respectively. Amplitudes of the feeding voltages can be varied independently. As a result the rectangular raster of the scanning beam was formed over the sample surface. Obviously, the electron flow was scattered in the gas and reached the sample surface as a wide spot rather than a thin beam.
Figure 2 presents the incident $\beta$-radiation power on 1 cm$^2$ of the sample surface calculated as a function of $x$- and $y$-distances from the sample center. The beam axis scans in $x$- and $y$-directions, the amplitudes of scanning being equal to $\pm 12$ cm. Figure 2(a) illustrates the bio-material treatment in helium at pressure 40 Torr and figure 2(b) – in water vapor at pressure 10 Torr. In the first case, four rigid peaks of the incident power appear at the raster corners and the sufficiently flat valley $\approx 5 \times 5$ cm$^2$ occurs between the peaks. The intensity of irradiation in the valley is about two times less than on the peaks. When the sample is treated in water vapor the flat plateau $\approx 7 \times 7$ cm$^2$ with uniform density of the $\beta$-radiation is formed on the sample surface.

![Figure 2](image1.png)

(a) (b)

**Figure 2.** The incident $\beta$-radiation power on 1 cm$^2$ of the sample surface calculated as a function of $x$- and $y$-distances from the sample center. The beam axis scans in $x$- and $y$-directions, the amplitudes of scanning being equal to $\pm 12$ cm; a – the treatment in the helium EBP at pressure 40 Torr, b – the treatment in the water vapor EBP at pressure 10 Torr.

![Figure 3](image2.png)

(a) (b)

**Figure 3.** The $\beta$-radiation power absorbed by a specific volume (1 cm$^2$ in square and thickness 1 $\mu$m) of the sample bulk as functions of the depth $L$ below the sample surface and the distance $R$ from the sample center. The EB scans in a circle, the circle radius in the sample plane being 5 cm; a – the treatment in the water vapor EBP, b – the treatment in the helium EBP.
The container with the treated powder (Petri cap 10 cm in diameter) was placed in front of the valley (in case of the treatment in the helium EBP) or in front of the plateau (in case of the treatment in the water vapor EBP). Since the density of the $\beta$-radiation power in the valley and on the plateau are approximately equal the same time periods are required to accumulate equal doses when the biomaterials are treated in helium and water vapor plasmas. The power of the injected EB is supposed to be constant.

Figure 3 presents the $\beta$-radiation power absorbed by a specific volume (1 cm$^2$ in square and thickness 1 $\mu$m) of the sample bulk as functions of the depth $L$ below the sample surface and the distance $R$ from the sample center. Both pairs of the deflecting coils are fed with equal sinusoidal voltages 50 Hz and, therefore, the EB scans in a circle. The circle radius in the sample plane is 5 cm. The simulation shows the power input to be maximal at depth $\approx 10$ $\mu$m under the sample surface and the penetration depth of the electrons into the treated material to be about 20 $\mu$m. Figure 3(a) illustrates the treatment in the water vapor EBP: the maximum of the $\beta$-irradiation income occurs at the sample centre ($R = 0$). In the helium EBP it occurs on the circumference $R = 3.5$ cm (see figure 3(b)).

7. The anti-aggregation activity of the FM modified by the EBP

To demonstrate the changes of biomaterial properties due to EBP-modification the products of FM treatment in the plasma of various gases were tested as platelet aggregation inhibitors. The model described in section 3 was used to calculate the dose of the irradiation and the real physical properties of FM were used to simulate the electron scattering by substrate.

The platelet aggregation $A$ (%) was measured by the turbidimetric method and $A$ was defined as the ratio of the light transparency of the platelet suspension after ceasing the aggregation process to the initial value of the light transparency [7]. The aggregation was monitored by the aggregometer Biola (Russia), adenosine diphosphoric acid ADP (final concentration $1\times10^{-5}$ M; Boechringer Mannheim, Germany) being used as an aggregation agent. The experimental data were statistically analyzed by Student’s test, P-values smaller than 0.05 were considered as reliable. Table 1 presents the results of the statistical analysis.

The water-soluble products of the treated FM inhibit the human platelet aggregation in vitro significantly whereas the native FM does not. Products of plasmachemically treated FM reduced the ADP-induced aggregation up to $\approx 33 – 35$ % in vitro at concentrations $1\times10^{-4}$-1 mg/ml, treatment in the water EBP being more effective than that in helium.

Table 1. The anti-aggregation activity of the FM modified by the EBP of water vapour and helium as a function of the concentration of the products in the studied solution.

| Plasma forming gas | Control ADP $1\times10^{-5}$M | Concentration of the products of the FM-modification in the solution, the anti-aggregation activity of which was measured, mg/ml |
|--------------------|-------------------------------|-----------------------------------------------------------------|
| H$_2$O             | 49.2±2.4                      | 35.2±1.9 35.5±1.9 33.3±1.4 33.2±1.7 38.0±2.3 42.6±1.7          |
| He                 | 55.3±1.9                      | 36.6±0.4 40.5±1.3 40.4±1.4 41.4±1.4 43.5±1.6 47.1±1.4          |

8. Conclusions

- The EBP treatment was experimentally proved to modify physical-chemical and biological properties of proteins. In particular, the fibrin-monomer acquires the anti-aggregation activity in-vitro for human platelets due to the treatment in the EBP of water vapor and helium.
• The plasmachemical processes, β-irradiation, UV- and X-ray radiations are jointly responsible for the modification effect.

• The plasmachemical processes are predominant, whereas the contribution of other actions mentioned above is of secondary importance.

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