Global diffusion limitations during the initial phase of the formation of a protein corona around nanoparticles

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Abstract

Herein, I illustrate analytically how the global diffusion limitations can influence the first phase of the protein-corona formation at nanoparticles under conditions of intravascular injection. In particular, the concentrations of proteins near the boundaries of the injection region are shown to be comparable with those far from the region. In contrast, the concentrations of proteins inside the injection region may be dramatically smaller than those outside, and the ratio of these two concentrations for proteins of different size may be much higher, by a few orders of magnitude, for smaller proteins. These differences in the spatial distribution of proteins are expected to play a key role in the Vroman effect at the onset of the protein-corona formation.

Keywords Nanoparticles · Protein corona · Diffusion limitations · Injection

1 Introduction

Injection of nanoparticles (NPs) into biofluids, e.g., to blood, is accompanied by attachment of proteins. This phenomenon known as the protein-corona (PC) formation is rich with fundamental questions to explore and of high current interest from the perspective of various applications, and the past ten years have witnessed explosive growth of the research conducted in this area (reviewed in [1–3]). Due to the diversity of proteins, their attachment is competitive. The specifics of this competition is that the whole process starts by adsorption of small proteins with the highest diffusion and attachment rates but eventually they are replaced by proteins with larger size and binding energy (Vroman effect). In addition, the attachment of proteins can be accompanied by their denaturation. Both these features of protein adsorption have long been well known in the conventional colloid and interface science [4, 5], and their importance in the context of the PC formation around NPs is often

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acknowledged in experimental studies [1, 2], mean-field kinetic models [6–10], and molecular dynamics simulations ([11–13] and references therein). Despite the apparent abundance of experimental and theoretical studies of the PC formation around NPs, the understanding of the mechanistic details of this process is still incomplete.

Focusing here on the initial phase of the PC formation, I may note that the available mean-field kinetic models [6–10] describe the competitive adsorption of proteins (Vroman effect) at NPs at the level of the difference of the attachment and detachment rate constants of small and large proteins. The effect of the local gradients of the protein concentrations around NPs on these rate constants is admitted, but globally the concentrations are assumed to be constant. Following this line, taking into account that the initial phase of the PC formation is often controlled by protein diffusion or, more specifically, by diffusion around NPs, and assuming the NPs to be spherical, one can use the seminal Smoluchowski expression for the adsorption rate,

$$W = 4\pi R D c_o, \quad (1)$$

where R is the NP radius, and c_o and D are the protein concentration and diffusion coefficient, respectively. According to hydrodynamics, one also has

$$D = \frac{k_B T}{6\pi\eta a}, \quad (2)$$

where a is the protein radius, and η is the solution viscosity coefficient. The time scale corresponding to appreciable coverage of a NP by protein in the absence of other proteins can be estimated by equating the protein uptake and the number of proteins needed to cover half of the NP surface, i.e., $4\pi R D c_o \tau \simeq 2\pi R^2 / \pi a^2$. Using this condition and expression (2), one gets

$$\tau = \frac{3R\eta}{ac_o k_B T}. \quad (3)$$

In this framework, the adsorption rate is proportional to $1/a$, and accordingly the corresponding rates of the increase of the NP coverage and the PC mass are proportional to a and a^2 . Thus, at comparable concentrations, the adsorption rate is indeed higher for smaller proteins (in agreement with the Vroman concept), but this effect is relatively weak because the difference in D is not appreciable especially if one compares it with the difference in concentration of various proteins, which is of the orders of magnitude in plasma. Moreover, the adsorption of the larger proteins is in fact dominating if one is interested in the NP coverage and PC mass.

The conventional arguments presented in the paragraph above should, however, be taken into account with reservation, because the initial phase of the PC formation around NPs is usually accompanied by the appearance of the gradients in the protein concentration not only locally on the nanoscale around NPs [as implied in the derivation of (1)] but also globally on the macroscopic length scale. Practically, this means that the protein concentration, c_o , introduced above should be identified with the local concentration, $c(x, y, z, t)$, which may be much lower compared to the initial concentration or the concentration far from the injection region. In the human blood plasma, for example, the dominant protein is Human Serum Albumin (HSA) with concentration $3.6 \times 10^{17} \text{ cm}^{-3}$ ($\simeq 40 \text{ g/l}$). Using this concentration in combination with $a = 3.5 \text{ nm}$, $R = 20 \text{ nm}$, and $\eta = 0.01 \text{ g/(s}\cdot\text{cm)}$ in (3), one obtains $\tau \simeq 10^{-5} \text{ s}$. This time scale is extremely short even compared to that of injection, and accordingly the global diffusion limitations related to the initial distribution of proteins and NPs cannot be ignored, because the time scale of the transition to the steady state without appreciable gradients in the protein concentration is much longer.

In conventional colloid and interface science, the role of global diffusion limitations in the initial phase of the protein adsorption on a macroscopic flat surface in general and in the Vroman effect in particular is well acknowledged [5, 14]. In contrast, experimental and theoretical studies of the PC formation around NPs do not pay attention to this aspect (reviewed in [3]). In particular, the available mean-field kinetic models [6–10] and molecular dynamics simulations [11–13] focused on the PC formation, the models scrutinizing NP motion in blood channels [15, 16], and the pharmacokinetic models describing the NP distribution in the organs at the compartment level [17] do not take it into account. Below, I present the 1D, 2D, and 3D versions of the simplest solvable generic model illustrating the likely effect of the global diffusion limitations on the protein uptake by NPs during the initial phase (just after injection) of the PC formation, i.e., during the onset of the formation of the adsorbed overlayer (the 1D version of this model was mentioned but not scrutinized in review [3]). At this stage, the diffusion-limited protein attachment to NPs occurs in parallel and the mutual influence of different proteins can be neglected. Despite this narrow focus (the subsequent replacement of proteins in the overlayer is not treated), the results presented clarify what may happen in the beginning and are instructive from this perspective.

2 1D model

On the macroscopic length scale, the evolution of the gradients of protein concentration depends on the conditions of measurements. In experiments performed *in vivo* and aimed at drug delivery, for example, direct intravascular injection of NPs is now the most useful route for administration [18, 19]. In the simplest generic 1D model illustrating the evolution of protein concentration in this important case, one can consider that initially (i) NPs are located in the injection region at $-l/2 \leq x \leq l/2$ (l is the corresponding macroscopic length scale), (ii) this region does not contain proteins, and (iii) the protein distribution at $x \leq -l/2$ and $x \geq l/2$ is uniform and their concentration there is equal to c_o [note that this definition of c_o is not fully identical to that used in the paragraph containing Eqs. (1)–(3)]. Focusing on diffusion in and near the injection region and taking into account that this region is usually small, the area outside can be considered to be infinite. Neglecting the blood flow or considering that the blood flow just shifts the whole region and neglecting the effect of the protein attachment to NPs on the protein-concentration profile (this is possible provided the NP concentration in the injection region is low), the evolution of protein concentration can be described by using the conventional diffusion equation. The corresponding text-book solution of this equation is as follows

$$c(x, t) = \frac{c_o}{2} \left\{ \operatorname{erfc} \left[\frac{-2x + l}{4(Dt)^{1/2}} \right] + \operatorname{erfc} \left[\frac{2x + l}{4(Dt)^{1/2}} \right] \right\}, \quad (4)$$

where $\operatorname{erfc}(x) \equiv (2/\pi^{1/2}) \int_x^\infty \exp(-z^2) dz$. The protein uptake calculated per NP located at position x in or near the injection region can then be represented as

$$U(x, t) = 4\pi DR \int_0^t c(x, \tau) d\tau. \quad (5)$$

The concentration profiles and uptake defined by (4) and (5) depend on the dimensionless parameter,

$$p \equiv (Dt)^{1/2}/l. \quad (6)$$

These profiles calculated at different values of $(Dt)^{1/2}/l$ (Fig. 1) can be considered to correspond to one kind of protein at different times or to different proteins at a given time. Bearing in mind different proteins, one can see that initially [at small values of $(Dt)^{1/2}/l$ as shown] the protein concentration at $x = \pm l/2$ is approximately equal to one half of that far from the injection region. This is also clear directly from (4), because at $x = \pm l/2$ the error function is either equal to 1 or close to zero, and accordingly

$$c(l/2, t) = c(-l/2, t) \simeq c_o/2, \quad \text{and} \quad (7)$$

$$U(l/2, t) = U(-l/2, t) \simeq 2\pi DRc_o t. \quad (8)$$

In contrast, the concentration near $x = 0$ is much smaller than c_o and strongly depends on p . For the latter region or, more specifically, at $x = 0$, Eqs. (4) and (5) yield

$$c(0, t) = c_o \operatorname{erfc} \left[\frac{l}{4(Dt)^{1/2}} \right] \simeq \frac{4c_o}{l} \left(\frac{Dt}{\pi} \right)^{1/2} \exp \left(-\frac{l^2}{16Dt} \right), \quad \text{and} \quad (9)$$

$$U(0, t) \simeq 256\pi^{1/2} c_o R l^2 \left(\frac{Dt}{l^2} \right)^{5/2} \exp \left(-\frac{l^2}{16Dt} \right). \quad (10)$$

The latter expression shows that during the first phase of the PC formation in the central area of the injection region the dependence of the protein uptake on D is exponentially strong.

To illustrate the scale of the effects predicted by Eqs. (6), (9), and (10), it is instructive to show p [Eq. (6)] as a function of l (Fig. 2) for $t = 1$ min and $D = D_{\text{HSA}} = 6.1 \times 10^{-7}$ cm²/s (this value corresponds to HSA). For practically reasonable sizes, $l \geq 1$ mm (this size correspond to typical volumes of the solution injected), p is large and accordingly the global diffusion limitations are expected to be appreciable. This conclusion is explicitly illustrated in Fig. 3 exhibiting the normalized uptake as a function of time at $x = 0$ with $l = 1$ mm for HSA ($D = D_{\text{HSA}}$) and smaller and larger proteins ($D = 1.5D_{\text{HSA}}$ and $D = D_{\text{HSA}}/1.5$), respectively. The difference in the uptakes is seen to be a few orders of magnitude.

Fig. 1 Initial and three transient protein-concentration profiles for $(Dt)^{1/2}/l = 0, 0.1, 0.15$, and 0.2 , respectively

Fig. 2 Diffusion-related dimensionless parameter, $p \equiv (Dt)^{1/2}/l$, as a function of the size of the injection region for $t = 1$ min and $D = D_{\text{HSA}} = 6.1 \times 10^{-7} \text{ cm}^2/\text{s}$

3 2D model

The simplest means of deriving expression (4) is convolution of the Green function for the 1D diffusion equation with the initial protein distribution. This approach can be used in the 2D and 3D cases as well. For the cylindrical geometry (2D case), one can consider that initially (i) NPs are located in the injection region at $r \equiv (x^2 + y^2)^{1/2} \leq \mathcal{R}$ (r is the radial coordinate, and $\mathcal{R}$ is the domain radius), (ii) this region does not contain proteins, and (iii) the protein distribution at $r \geq \mathcal{R}$ is uniform and their concentration there is equal to c_0 . Neglecting again (cf. Section 2) the blood flow and the effect of the protein attachment to NPs on the protein-concentration profile, the evolution of protein concentration can be described as

$$c(x, y, t) = \int_{r \geq \mathcal{R}} G(x - x', y - y', t) c(x', y', 0) dx' dy', \quad (11)$$

where

$$G(x, y, t) = \frac{\exp[-(x^2 + y^2)/4Dt]}{4\pi Dt} \quad (12)$$

is the Green function. Qualitatively, the evolution of the protein concentration is in this case similar to that in the 1D case. In particular, employing (11) in combination with (12), one can easily obtain that the concentration near $r = 0$ is given by

$$c(0, t) = c_0 \exp(-\mathcal{R}^2/4Dt). \quad (13)$$

Fig. 3 Normalized protein uptake per nanoparticle as a function of time at $x = 0$ and $l = 1$ mm [according to Eq. (10)] for HSA ($D = D_{\text{HSA}}$; line 1) and smaller and larger proteins with $D = 1.5D_{\text{HSA}}$ (line 2) and $D = D_{\text{HSA}}/1.5$ (line 3), respectively

Initially (at $t < \mathcal{R}^2/4D$), this concentration strongly depends on t and is much smaller than c_o . In this limit, the corresponding protein uptake defined in analogy with (5) is given by

$$U(0, t) \simeq 16\pi c_o R \mathcal{R}^2 \left(\frac{Dt}{\mathcal{R}^2} \right)^2 \exp \left(-\frac{\mathcal{R}^2}{4Dt} \right). \quad (14)$$

4 3D model

For the spherical geometry (3D case), one can consider that initially (i) NPs are located in the injection region at $r \equiv (x^2 + y^2 + z^2)^{1/2} \leq \mathcal{R}$, (ii) this region does not contain proteins, and (iii) the protein distribution at $r \geq \mathcal{R}$ is uniform and their concentration there is equal to c_o . With this specification, Eqs. (11)–(13) can be rewritten as

$$c(x, y, z, t) = \int_{r \geq \mathcal{R}} G(x - x', y - y', z - z', t) c(x', y', 0) dx' dy' dz', \quad (15)$$

$$G(x, y, z, t) = \frac{\exp[-(x^2 + y^2 + z^2)/4Dt]}{(4\pi Dt)^{3/2}}, \quad (16)$$

$$c(0, t) = \frac{c_o}{(4\pi Dt)^{3/2}} \int_{\mathcal{R}}^{\infty} \exp(-r^2/4Dt) 4\pi r^2 dr. \quad (17)$$

Initially (at $t < \mathcal{R}^2/4D$), one has

$$c(0, t) \simeq \pi^{-1/2} c_o \left(\frac{\mathcal{R}^2}{Dt} \right)^{1/2} \exp \left(-\frac{\mathcal{R}^2}{4Dt} \right). \quad (18)$$

Fig. 4 Protein uptake per nanoparticle (normalized to $U_* \equiv R\mathcal{R}^2 c_o$) as a function of time (normalized to $t_* \equiv \mathcal{R}^2/4D$) in the 1D, 2D, and 3D cases at $x = 0$ or $r = 0$ according to Eqs. (20), (14), and (19), respectively

The corresponding uptake is given by

$$U(0, t) \simeq 16\pi^{1/2} c_o R\mathcal{R}^2 \left(\frac{Dt}{\mathcal{R}^2} \right)^{3/2} \exp \left(-\frac{\mathcal{R}^2}{4Dt} \right). \quad (19)$$

5 Conclusion

In summary, the results presented clearly indicate that the global diffusion limitations can play a crucial role during the first phase of the PC formation around NPs under conditions of intravascular injection. Although this conclusion is what one could expect (cf. the Introduction), its scrutiny, description of the protein-concentration profiles, and illustration of the influence of the diffusion limitations on the protein uptake are novel and instructive. In particular, the concentrations of proteins near the boundaries of the injection region are shown to be comparable with those far from the region. In contrast, the concentrations of proteins inside the injection region may be dramatically smaller than those outside, and their ratio may be much higher, by a few orders of magnitude, for smaller proteins. The latter feature determines the PC composition at the onset of the PC formation around NPs and accordingly is expected to play a key role in the Vroman effect during the subsequent phases the PC formation. In other words, this means that the composition of the PC may be very different from the very beginning of the PC-formation process, and it may influence what happens afterwards.

The conclusions above are applicable irrespective of the dimensionality. To illustrate the role of the dimensionality, it is instructive to compare the uptakes predicted by Eqs. (10),

(14), and (19) corresponding to the 1D, 2D, and 3D cases, respectively. Equations (14) and (19) contain $\mathcal{R}$ (one half of the injection-region size) and can be compared directly, while (10) contains l (injection-region size). For direct comparison with (14) and (19), one can identify l with $2\mathcal{R}$ and rewrite (10) as

$$U(0, t) \simeq 32\pi^{1/2} R \mathcal{R}^2 c_0 \left(\frac{Dt}{\mathcal{R}^2} \right)^{5/2} \exp \left(-\frac{\mathcal{R}^2}{4Dt} \right). \quad (20)$$

With this modification, one can see (Fig. 4) that as expected the uptake increases with increasing dimensionality.

Finally, I repeat (cf. the Introduction) that in experimental studies of the PC formation around NPs after injection (see, for example, Refs. [19–27]) the very initial phase of this process is usually not scrutinized. For this reason, I do not use the results obtained here for the interpretation of specific experiments.

Compliance with Ethical Standards

Conflict of interests The author declares that he has no conflict of interest.

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