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Grey Theory Based Analysis of Factors Affecting Cellular Uptake in Nano-particle Drug System

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Abstract

The Grey correlation degree among the cellular uptake and the features of drug particle is studied, the concerned features include the shape, size, ζ- potential of the drug particle. The grey correlation degrees for above factors are calculated and analyzed on basis of some experimental data from available literature. The results showed that the effect of surface ζ-potential is the most significant in the process of cellular uptake, followed by the factor of the particle shape, while the effect of particle size sits the third one.

Keywords: Grey correlation degree, Cellular uptake, ζ-potential, Particle size, Particle shape.

Introduction

The shape, size and surface chemistry of nano-particle drug have a crucial influence on drug delivery in vivo absorption and metabolism, and the effect of above factors is quite different. Therefore, the analysis of the role of above factors has practical significance for the optimal design of nano-particle drug[1]. The successful stretching of polystyrene spheres into particles with a wide array of geometries has enabled Champion et al to perform their pioneering work [2], their experimental results demonstrate that the local shape of particle is the major factor on whether simply spreading or phagocytosis occurs. By analyzing the effect of diversely shaped micro-sized polystyrene particles, including spheres, oblate and prolate ellipsoids, elliptical and rectangular disks as well as discoid, on macrophages over time, a dimensionless shape-dependent parameter related to the length normalized curvature, Ω, was defined (see diagram in Figure. 1A). The predisposition to phagocytosis and the internalization velocity of the particles as a function of Ω was also reported [2]. In essence, particles were found to be internalized successfully when $\Omega \leq 45°$ via actin-cup and ring formation, with phagocytosis velocity being inversely correlated to Ω (up to $45°$); on the other hand, when $\Omega > 45°$, cell spreads, but no internalization occurring. In contrast, the contribution of particle size or volume to the
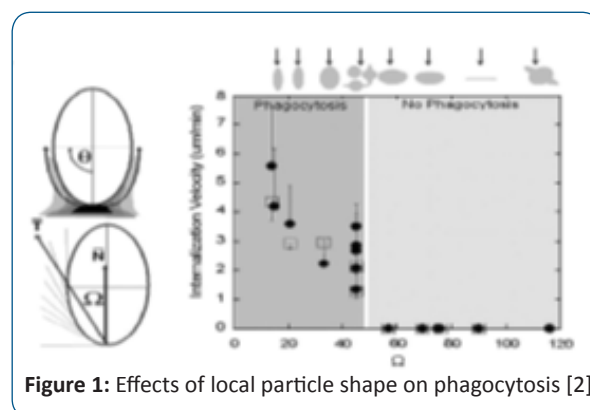

Figure 1: Effects of local particle shape on phagocytosis [2]

phagocytotic process was evidently lower as compared to particle shape, it affects the completion of particle internalization only when the particle volume is greater than that of the macrophage at $\Omega \leq 45°$.

Particle shape, together with size, was also found to affect their internalization by non- phagocytic cells. In one systematic study, Gratton et al fabricated a series of cationic cross-linked PEG-based hydrogels of varying sizes and shapes via the top-down lithographic PRINT technique and examined the extent, rate and mode of cellular internalization of the particles using HeLa cells [2,5,6].

As a comparison, nanometer-sized cylinder was found to be internalized to the greatest extent, followed by the larger micrometer-sized cylinders and lastly, the cubic-shaped particles (Figure 2). It was further demonstrated that it discovered that the internalization kinetics of the nanometer-sized cylinders in HeLa cells is affected by the particle aspect ratio (AR) and volume in addition to the shape. These results indicate that it found quite different rates of internalization for cylinders with 1) higher AR, but same volume or 2) larger volume, but same aspect ratio. The greater internalization of the cylindrical particles is speculated to be due to their larger surface area, which allows more multivalent
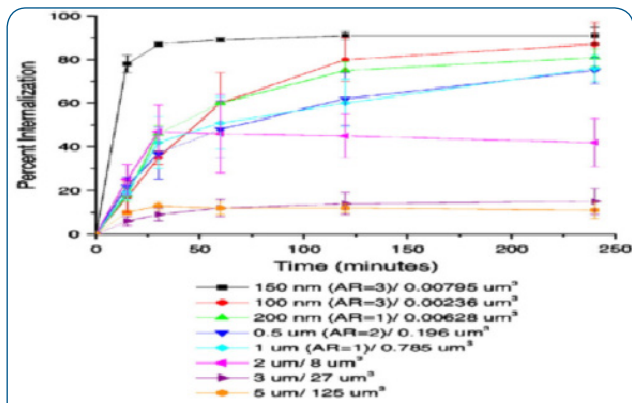
Figure 2: Internalization profiles of cylindrical and cubic-shaped PRINT particles of various sizes in HeLa cells over a 4 h incubation period at 37 ˚C [2-4]

ionic interactions with the cell membranes to undergo clarthrin- and caveolae- mediated endocytosis as well as to a lesser extent, macropinocytosis.

Marianne Roser et al used ζ-potential to describe the effect of modified surface [7]. They found a good correlation between stimulation of macrophages and the ζ-potential measured by electrophoretic mobility. The less the surface charge of particles, the lower the phagocytic activity of U-937 [7]. An increase in phagocytic response was observed by using particles with a high surface potential, especially a high positive net charge, see Figure 3. Different surface chemistry for drug results in different surface electric charge. The phagocytic cell of different positive and negative charge exhibits quite different [7-11].
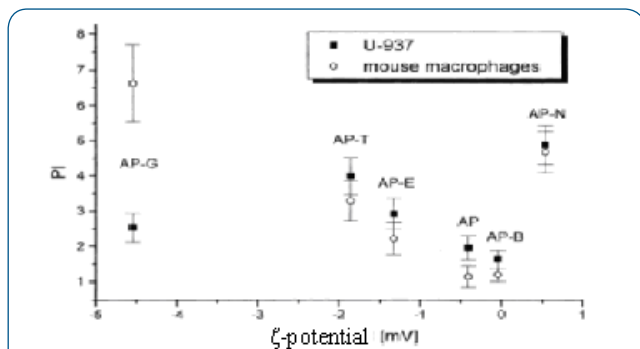

Figure 3: Correlation between phagocytic index and ζ-potential (phosphate buffer, 5 mM, pH 7.2) of surface modified albumin nanoparticles using U-937 cells (incubation time 60 min, presence of human serum) and mouse macrophages (incubation time 60 min, without serum) [7]

Shityakov et al attempted to predict and emphasize molecular interactions of dopamine, levodopa, and their derivatives (Dopimid compounds) containing 2-phenyl-imidazopyridine moiety with the α-cyclodextrindimmer [12],so as to assess and improve drug delivery to the central nervous system. The molecular docking method was used to determine the energetic profiles, hydrogen bond formation, and hydrophobic effect of 14 host–guest complexes. The results showed that the "chemical branching" represented by additional ethyl-acetate residue is energetically unfavorable and promotes a conformational shift due to the high root mean square deviation levels. This phenomenon was characterized by a low number of H-bonds and

a significant decrease of the host–guest hydrophobic potential surface. Shityakov et al also studied the ability of a multiwalled carbon nanotube functionalized with fluorescein isothiocyanate (MWCNT–FITC) by assessing it as a prospective central nervous system-targeting drug delivery system to permeate the blood–brain barrier [13]. The results indicated that the MWCNT–FITC conjugate was able to penetrate microvascular cerebral endothelial monolayers; its concentrations in the Transwell® system were fully equilibrated after 48 hours. Cell viability test, together with phase-contrast and fluorescence microscopies, did not detect any signs of MWCNT–FITC toxicity on the cerebral endothelial cells. Colilla et al. prepared mesoporous silica- zirconia mixed oxides, which was used as efficient drug delivery carriers for bisphosphonates based drugs via spray-drying process [14]. These silicazirconia mixed oxides used as drug delivery systems exhibit mesostructures with tunable acidity for the first time. The two selected drugs were alendronate and zoledronate, which carry similar phosphonate heads but different amine tails in terms of acidity and hydrophobicity. The incorporation of different zirconium amounts into the meso-structured silica network creates Lewis and Brönsted acid surface sites allowing to control the surface properties of the mesoporous matrix, and therefore the amount of phosphonate adsorbed into the mesopores. This effect was found even at low ZrO_2 contents and it induced a noticeable modification on the release behavior of bisphosphonates. The partial retention of the drug through phosphonatescomplexation by zirconium sites led to a more sustained release of the drug.

These results show that different factors have a crucial influence metabolism. These could be imaged in the role attributed to the relationship in Figure 4[1]. However, the relative importance of different factors of nano-particle drug on cellular uptake is not clear yet till now.
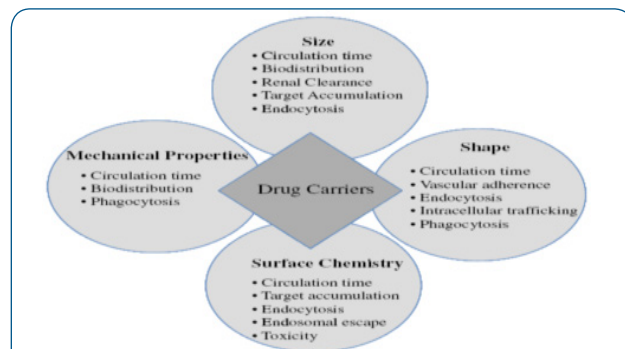

Figure 4: Role of key carrier properties in drug delivery

Gray theory was proposed by Deng Julong in 1980s, which emerges interdisciplinary [15-18]. The basic idea of Gray system theory is that it develops and extracts valuable information to achieve the behavior of the system mainly through the generation of some known information, and obtain a running correct description for the evolution of the system, though the whole system is in a status of "knowing some information, unknowing some information", the "small sample", "poor information", etc, and even uncertainty. Different factors, such as shape, size, surface chemistry and mechanical flexibility, etc. have a crucial influence on in vivo distribution, absorption, metabolism, etc. However,

it is not clear which factor plays an important role in promoting the development of the system. So, it needs to study the nature in light of grey theory correlation degree analysis. This paper aims to perform this analysis in the light of grey correlation analysis.

Analysis of Grey Correlation

Correlation among the relevant parameters

The basic idea of gray correlation analysis is to determine the closeness based on the sequence similarity of geometry of the curves [19,20]. The closer the curve, the greater degree of correlation among the corresponding sequences.

In order to perform the analysis for an event, it needs to reflect the features of the system accurately into a data sequence first, which is called "building the characters of the system by mapping", the mapping is an indirect measure to determine the behavior parameter of system.

In general, the rate of internalization has been employed to measure the speed of particles entering into cells, the size and shape of the particles are employed to represent the geometric features of drug particle, ζ-potential is a character to reflect the physicochemistrical feature of a drug particle surface. As long as the characteristic data of system is set up, the patterns of individual sequence could be made intuitively.

In Ref. [2,6] and Figure 1, the experimental data indicated the correlation between particle shape factor Ω and internalization rate, which is cited here in Table 1.

Refer to literature [2-4] and Figure 2, the percent internalization at 4 hours for different sized particle is cited here in Table 2.

While, From Ref. [7] and Figure 3, it extracted the variation of phagocytic index value vs ζ-potential, as is shown here in Table 3.

Table 1: Corresponding correlation of particle shape factor Ω with internalization rate

$\Omega°$	14	16	22	34	45
Internalization rate I (m/min)	5.5	4.0	3.4	2.2	1.2

Table 2: The percentage of different sizes of 4 hours [2-4]

Size (nm)	100	150	200	500	1000	2000	3000	5000
Percent internalization (%)	83	85	80	72	70	40	13	9

Table 3: The corresponding index values of different ζ-potentials in u-937

ζ-potential (mv)	-0.1	-0.4	-1.3	-1.9	-4.5
phagocytic index	1.5	1.8	3.0	4.0	2.5

Calculation of gray correlation

The internalization rate is the percent internalization, and the phagocytic index is the description of the status of drug particle entering into cells. According to grey theory [19,20], the above data can be rewritten as following form for the procedure of gray processing.

$Y_1 = \{1.2, 2.2, 3.4, 4.0, 5.5\}$, $X_1 = \{45, 34, 22, 16, 14\}$

$Y_2 = \{83, 85, 80, 72, 70\}$, $X_2 = \{100, 150, 200, 500, 1000\}$

$Y_3 = \{1.5, 1.8, 3.0, 4.0, 2.5\}$, $X_3 = \{-0.1, -0.4, -1.3, -1.9, -4.5\}$

Absolute correlation degree calculated by gray theoretic approach

Calculation of starting point for the sequences

$Y_1^0 = \{0, 1.0, 2.2, 2.8, 4.3\}$, $X_1^0 = \{0, -11, -23, -29, -31\}$

$Y_2^0 = \{0, 2, -3, -11, -13\}$, $X_2^0 = \{0, 50, 100, 400, 900\}$

$Y_3^0 = \{0, 0.3, 1.5, 2.5, 3.5\}$, $X_3^0 = \{0, -0.3, -1.2, -1.8, -4.4\}$

4.2.1.2 Calculations for the grey based absolute value

$$\left|Y_{s1}\right| = \left|\sum_{k=2}^{4} Y_1^0(k) + \frac{1}{2} Y_1^0(5)\right| = 8.5$$

$$\left|X_{s1}\right| = \left|\sum_{k=2}^{4} X_1^0(k) + \frac{1}{2} X_1^0(5)\right| = 78.5$$

$$\left|X_{s1} - Y_{s1}\right| = \left|\sum_{k=2}^{4}[X_1^0(k) - Y_1^0(k)] + \frac{1}{2}[X_1^0(5) - Y_1^0(5)]\right| = 86.065$$

Calculation of the gray based correlation degree

$$\varepsilon_{11} = \frac{1 + \left|X_{s1}\right| + \left|Y_{s1}\right|}{1 + \left|X_{s1}\right| + \left|Y_{s1}\right| + \left|X_{s1} - Y_{s1}\right|} = \frac{87.65}{174.30} = 0.5029$$

Similarly, the grey based absolute correlation for the remaining parameters can be calculated as well, it obtains,

$\varepsilon_{11} = 0.5029$, $\varepsilon_{12} = 0.5090$, $\varepsilon_{13} = 0.5171$

$\varepsilon_{21} = 0.6203$, $\varepsilon_{22} = 0.5002$, $\varepsilon_{23} = 0.6579$

$\varepsilon_{31} = 0.5030$, $\varepsilon_{32} = 0.5026$, $\varepsilon_{33} = 0.5231$

Furthermore, it gets the matrix for absolute correlation degree

$$A = (\varepsilon_{ij}) = \begin{bmatrix} 0.5029 & 0.5090 & 0.5171 \\ 0.6203 & 0.5002 & 0.6579 \\ 0.5030 & 0.5026 & 0.5231 \end{bmatrix}$$

Analysis and discussion

From the matrix A of the absolute correlation degree, the element of each row meets basically, $\varepsilon_{i3} > \varepsilon_{i2} > \varepsilon_{i1}$, $i=1, 2, 3$; So the effect of affecting factor X_3 is the more significant than X_1 and X_2. While in ε_{i1}, there are 2 numbers which are bigger than that in ε_{i2}, but for the summation, the result $\sum_{i=1}^{3} \varepsilon_{i1} > \sum_{i=1}^{3} \varepsilon_{i2}$ holds, therefore, X_1 takes priority over X_2 in whole. Thus, above analysis indicates that the effect of particle surface charge is the main affecting factor in the process of cellular uptake and phagocytosis, followed by the effect of shape of drug particle, and the effect of drug particle size is relatively lower. Furthermore, the study implies that the tip of drug particle is the prefer position for cellular uptake due to the tip effect of electric charge from static electricity, it has been proved by many experiments [2]. The influence of particle size

is effective as well, the smaller the particle size, the more uptake the cell.

Conclusion

This paper performed a grey theory based analysis for the effect of nano- particle drug features on cellular uptake process, including the particle shape, size and surface charge.Analysis showed that surface charge plays a greater role in the process of cellular uptake process,followed by the shape and size of drug particle. Therefore, more attention should be paid to make the drug particle to have higher ζ-potential in the drug particle design and preparation, which will make uptake of drug particle more effectively.

Acknowledgments

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