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ΦΑΡΜΑΚΕΥΤΙΚΗ
PHARMAKEFTIKI

ΤΡΙΜΗΝΙΑΙΑ ΕΚΔΟΣΗ ΜΕ ΘΕΜΑΤΑ ΦΑΡΜΑΚΕΥΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
A QUARTERLY EDITION ON PHARMACEUTICAL SCIENCES' TOPICS

ΗΜΕΡΙΔΑ ΝΑΝΟΤΕΧΝΟΛΟΓΙΑΣ ΣΤΙΣ ΕΠΙΣΤΗΜΕΣ ΥΓΕΙΑΣ

SYMPOSIUM ON NANOTECHNOLOGY IN HEALTH SCIENCE

ΤΟΜΟΣ VOLUME 29 | ΤΕΥΧΟΣ ISSUE IV

ΟΚΤΩΒΡΙΟΣ - ΔΕΚΕΜΒΡΙΟΣ OCTOBER - DECEMBER 2017

ΕΛΛΗΝΙΚΟΣ
ΣΥΝΔΕΣΜΟΣ
ΤΟΥΡΙΣΜΟΥ ΥΓΕΙΑΣ

Η σύνδεση της «βιομηχανίας» Τουρισμού με την Υγεία γίνεται πραγματικότητα

Ιδρύθηκε και ξεκίνησε άμεσα την δραστηριότητα του ο **Ελληνικός Σύνδεσμος Τουρισμού Υγείας**.

Δημιουργήθηκε από επιφανή μέλη της τουριστικής βιομηχανίας και έγκριτους επιστήμονες υγείας, οι οποίοι διέγνωσαν τα σημαντικά πλεονεκτήματα και την προστιθέμενη αξία που μπορεί να έχει ο Τουρισμός Υγείας, στον Ελληνικό Τουρισμό, στον κλάδο της Υγείας και επιπρόσθετα στην Οικονομία και το ΑΕΠ της χώρας.

Σκοπός του Συνδέσμου είναι **η σύνδεση, ανάπτυξη και διεύρυνση της «βιομηχανίας» του τουρισμού, με το πολύτιμο αγαθό της υγείας**, μέσα από την συνεχή υποστήριξη και προβολή των παρακάτω κατηγοριών:

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ΤΟΜΟΣ 29, ΤΕΥΧΟΣ IV,
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Editorial note

Το τεύχος αυτό του περιοδικού «ΦΑΡΜΑΚΕΥΤΙΚΗ» αποτελεί ειδικό τεύχος, αφιερωμένο στη δημοσίευση σύντομων άρθρων των ομιλιών που έχουν επιλεγεί να παρουσιαστούν στην επιστημονική συνάντηση με τίτλο «Νανοτεχνολογία στις επιστήμες της υγείας» που διοργανώνει η Ελληνική Εταιρεία Νανοτεχνολογίας στις Επιστήμες Υγείας (ΕΛΕΝΕΠΥ) στα πλαίσια του 2ου Ελληνικού Συνεδρίου Φαρμακευτικής περίθαλψης στις 21 Ιανουαρίου 2018. Ευχαριστούμε ιδιαιτέρως την αρχισυντάκτη του περιοδικού, καθηγήτρια κ. Άννα Τσαντίλη και την επιτροπή σύνταξης του περιοδικού για την ευκαιρία που μας έδωσαν να παρουσιάσουμε σε αυτό το τεύχος, σε ένα ευρύτερο κοινό, προηγμένα θέματα νανοϊατρικής παρουσιασμένα από εξαιρετικούς επιστήμονες, οι οποίοι εργάζονται ερευνητικά σε καινοτόμους τομείς των επιστημονικών τους πεδίων.

Τα μέλη του Διοικητικού Συμβουλίου της ΕΛΕΝΕΠΥ είναι:
- Πρόεδρος, Ευστάθιος Π. Ευσταθόπουλος, Καθηγητής, Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών (ΕΚΠΑ)
- Αντιπρόεδρος, Κωνσταντίνος Ν. Δεμέτζος, Καθηγητής, ΕΚΠΑ
- Γεν. Γραμματέας, Σπυρίδων Κίντζιος, Καθηγητής, Γεωπονικό Πανεπιστήμιο Αθηνών
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- Αναπλ. Μέλος, Πηνελόπη Μπουζιότη, Ερευνήτρια Α', ΕΚΕΦΕ «Δημόκριτος»
- Αναπλ. Μέλος, Γιώργος Θεοδωρόπουλος, Αναπλ. Καθηγητής, ΕΚΠΑ
- Αναπλ. Μέλος, Αγάπη Πλουσή, Υποψ. Διδάκτωρ, ΕΚΠΑ

This special issue of "PHARMAKEFTIKI" is dedicated to publish short articles of the talks that have been selected for presentation in "Nanotechnology in Health Sciences", a session organized by the Hellenic Society of Nanotechnology in Health Sciences (HSnanoHS) in the framework of the 2nd Hellenic Congress of Pharmaceutical care on the 21st of January 2018. We owe special thanks to the editor, Prof. Anna Tsantili, and the editorial board of the journal for this opportunity to present to a wider audience state-of-the-art nanomedicine topics by excellent scientists working in the scientific front-end of the field.

The members of the Executive Committee (ExCo) of HSnanoHS are :
- President, Efstathios P. Efstathopoulos, Professor, National and Kapodistrian University of Athens (NKUA)
- Vice President, Constantinos N. Demetzos, Professor, NKUA
- General Secretary, Spyridon Kintzios, Professor, Agricaltural University of Athens (AUA)
- Treasurer, George Loudos, Assistant Professor, Technological Educational Institute of Athens
- Member, Maria Gazouli, Asociate Professor, NKUA
- Assoc. Member, Penelope Bouziotis, Senior Researcher, NCSR "Democritos"

About Nanotechnology and the Hellenic Society of Nanotechnology in Health Sciences (HSnanoHS)

Nanotechnology is a promising multidisciplinary scientific field that deals with the development and use of materials with dimension equal to one billionth of a meter. It is a scientific discipline that has attained a huge and heavy-weight status in science and technology and has attracted the attention to those working in health sciences and in the environmental scientific fields.

This special issue includes extended abstracts and short papers regarding drug delivery systems, imag-

ing and diagnostic approaches as well as the developing and evaluating challenges of nanomaterials for producing health care final products. The applications of innovative nanosystems and nanodevices as drug delivery platforms or as tools in diagnosis and imaging are presented. It is our belief that the application of principles of nanotechnology and the development of nanotechnological products, could be emerged as the building block for designing better medicines and improving the health.

Based on the above, we have decided to establish a scientific institution, the Hellenic Society of Nanotechnology in Health Sciences (HSnanoHS) as it is dealing with the wider subject of nanotechnology in the area of health sciences. I will take the opportunity to make a short presentation of our society. HSnanoHS was established in Athens, Greece in December 2016. The society is a multidisciplinary body, consisting of scientists from different scientific areas.

The aims of our society are to:

- Advance knowledge in the field of Nanotechnology in Health Sciences;
- Promote and disseminate the research, educational and professional activities of its Members, at a national and international level;
- Promote collaboration, interaction and complementarity among its Members;
- Promote networking of key-actors in the field of Nanotechnology in Health Sciences, at a national level;
- Promote networking and collaboration of the Society with national and international scientific, research, funding and investment institutes;
- Promote collaborations between national and foreign institutes, active in the field of Nanotechnology in Health Sciences;
- Promote the common stand point of the Members of the Society to public authorities;
- Promote the resolution of issues concerning the legal/regulatory context applicable in connection with activities of research, development and business, in the field of Nanotechnology in Health Sciences;
- Promote activities supporting the increase of the availability of national funds for education, research, and development, in the field of Nanotechnology in Health Sciences.
- Any other activity relevant to the aims of the Society.

Any Physical or Legal Body activating in the field of Nanotechnology in Health Sciences is eligible to become a full member of the HSnanoHS.

Our founding members come from academia, research centers, public and private hospitals, and business sector.

Our society has organized the following scientific events:

1. A session included in "DYO Forum", titled "Applications of Nanotechnology in Health Sciences" in Athens, February 2017.

2. The 27th Interdisciplinary Research Conference on Injectable Osteoarticular Biomaterials and Bone Augmentation Procedures, in collaboration with the GRIBOI association, in Athens, May 2017.

Moreover, HSnanoHS has been participated in a number of scientific events:

- The 4th Athens Science Festival (29 March – 2 April 2017), one of the largest Greek outreach events, which targets general public.
- The 31st annual meeting of the European Society of Hyperthermic Oncology (ESHO 2017, 21 – 23 June 2017), in Athens.
- The 18th Hellenic Pharmaceutical Conference (6-8 October 2017) in Athens.
- The Conference on Bio-Medical Instrumentation and related Engineering and Physical Sciences, BIOMEP 2017, organized by the Technological Educational Institute of Athens, on 12 -13 October in Athens.

Our society has awarded the title of "honorary member of HSnanoHS" to a number of famous scientists in their fields; Prof. Erem Bilensoy, Hacettepe University Faculty of Pharmacy, Turkey, President of EUFEPS, Prof. Robert Ivkov, Johns Hopkins University School of Medicine, US, Prof. Francis Ligler, Joint NC State and UNC-Chapel Hill, US, Prof. Igor Sokolov, Tufts University, US and Dr. Charalambos Koutsoulas, Polymun Scientific, Austria, Assoc. Member, George Theodoropoulos, Associate Professor, NKUA, Assoc. Member, Agapi Ploussi, Ph.D. Candidate, NKUA.

Ημερίδα Νανοτεχνολογίας στις Επιστήμες Υγείας

21-1-2018, Μέγαρον Διεθνές Συνεδριακό Κέντρο Αθηνών

14:30 - 14:50
Καινοτόμα Νάνο-Εμβόλια: Από την Έρευνα στην Κλινική Πράξη
Ν. Πίππα, Μεταδιδακτορική Ερευνήτρια ΕΚΠΑ, Φαρμακοποιός, MSc, PhD
Συμμετέχει: Κωνσταντίνος Δεμέτζος

14:50 - 15:10
Έξυπνα Υβριδικά Πολυπεπτιδικά Νανοσωματίδια για τη Στοχευμένη Μεταφορά Δοξορουβικίνης
Ερμόλαος Ιατρού, Καθηγητής, Τμήμα Χημείας, ΕΚΠΑ

15:10 - 15:30
In Vitro Δράση Υπέρθερμικών Fe3O4 Νάνοσωματιδίων Ag και Au σε Καλλιέργειες Καρκινικών Κυττάρων
Άννα Λυμπεροπούλου, Δρ. Βιολόγος, Ιατρική Σχολή, ΕΚΠΑ
Συμμετέχουν: Βασίλης Κουλουλίας, Ievgen Pylypchuk, Iuliia Mukha, Liudmyla Storozhuk, Μ. Γαζούλη, Nadiia Vityuk, Στ. Γραμματικάκη

15:30 - 15:50
Folate-Positive Cancer Cell Biosensor Based on an Electroactive Hydrogel Doped With Silver Enhanced/Folic Acid Funtionalized Gold Nanoparticle-Targeted Cells
Δήμητρα Θεοδόση - Παλημέρη, Βιοτεχνολόγος, Γεωπονικό Παν. Αθηνών
Συμμετέχουν: Ευαγγελία Φλαμπούρη, Σπυρίδων Κίντζιος

15:50 - 16:00
Συζήτηση – Ερωτήσεις

16:00 - 16:10
Διάλειμμα – Snack

16:10 - 16:30
Η Νανοϊατρική στη Διάγνωση και Θεραπεία Νευρολογικών Παθήσεων
Ευστάθιος Π. Ευσταθόπουλος, Καθηγητής ΕΚΠΑ, Πρόεδρος ΕΛΕΝΕΠΥ
Συμμετέχει: Αγάπη Πλουσή

16:30 - 16:40
Ανάπτυξη Νανοσυστημάτων Λυοτροπικών Υγρών Κρυστάλλων για την Μεταφορά Θεραπευτικών Παραγόντων
Μαρία Χουντουλέση, Υποψήφια Διδάκτωρ, Φαρμακοποιός, MSc
Συμμετέχουν: Κ. Δεμέτζος, Ν. Πίππα, Ν. Ταβερναράκης, Σ. Πίσπας

16:40 - 16:50
Φυσικοχημικά Χαρακτηριστικά των Λιποσωμάτων και η Επίδραση του Λυοτροπισμού τους στην Πρωτεϊνική Σύνδεση in Vitro
Φωτεινή Παπαγεωργίου, Φαρμακοποιός
Συμμετέχουν: Κωνσταντίνος Δεμέτζος, Ν. Πίππα, Ν. Ναζίρης

16:50 - 17:00
Ο Ρόλος της Σχέσης Πληροφορίας / Εντροπίας στην Αυτο-οργάνωση Χιμαιρικών Νανοσυστημάτων Μεταφοράς Φαρμακομορίων
Νικόλαος Ναζίρης, Υποψήφιος Διδάκτωρ, Φαρμακοποιός, MSc
Συμμετέχουν: Κωνσταντίνος Δεμέτζος, Νατάσσα Πίππα, Στέργιος Πίσπας

17:00 - 17:10
Ανασκόπηση στις Νέες Εξελίξεις στη Θεραπεία του Καρκίνου Στηριζόμενες στη Διπλή Δράση των Νανοσωματιδίων Χρυσού
Ελλάς Σπυράτου, Ph.D., Μεταδιδακτορική Ερευνήτρια, Β' Εργαστήριο Ακτινολογίας, Ιατρική Σχολή ΕΚΠΑ
Συμμετέχουν: Ε. Ευσταθόπουλος, Μ. Μακροπούλου, Α. Γεωργακίλας

17:10 - 17:30
Συζήτηση – Ερωτήσεις

One - Day - Symposium on Nanotechnology in Health Science

21-1-2018, Megaron International Congress Center

14:30 - 14:50
Innovative nano-vaccines: From bench to bedside
Natassa Pippa , post Doc Researcher in NKUA
Contributors: Costas Demetzos

14:50 - 15:10
Smart Hybrid Polypeptide-Containing Nanoparticles for the Targeted Delivery of Doxorubicin
Hermis Iatrou, Professor, Department of Chemistry, NKUA
Contributors: Panayiotis Bilalis, Leto-A. Tziveleka, Spyridon Varlas

15:10 - 15:30
In vitro effect of hyperthermic Ag and Au Fe3O4 nanoparticles in cancer cell cultures
Anna Lyberopoulou, Dr Biologist, School of Medicine, NKUA
Contributors: S. Grammaticaki, Iu. Mukha, N. Vityuk, Ie. Pylypchuk, L. Storozhuk, B. Kouloulias, M. Gazouli

15:30 - 15:50
Folate-Positive Cancer Cell Biosensor Based on an Electroactive Hydrogel Doped With Silver Enhanced/Folic Acid Funtionalized Gold Nanoparticle-Targeted Cells
Dimitra Theodosi-Palimeri, Biotechnolgist, Agricultural University of Athens, Greece
Contributors:Evangelia Flampouri, Spyridon Kintzios

15:50 - 16:00
Discussion

16:00 - 16:10
Snack-break

16:10 - 16:30
Nanomedicine in diagnosis and treatment of neurological diseases. A literature review
Efstathios P. Efstathopoulos. Professor, NKUA, President of ΕΛΕΝΕΠΥ
Contributors: Agapi Ploussi

16:30 - 16:40
Developing therapeutic drug delivery nanosystems from different lyotropic liquid crystalline mesophases
Maria Chountoulesi, PhD student, Pharmacist, MSc
Contributors: Natassa Pippa, Nektarios Tavernarakis, Stergios Pispas, Costas Demetzos

16:40 - 16:50
Physicochemical characteristics of liposomes and their lyotropism influence for protein-liposome interactions in vitro
Foteini Papageorgiou, Pharmacist
Contributors: Natassa Pippa, Nikolaos Naziris, Costas Demetzos

16:50 - 17:00
The Role of the Information/Entropy Balance in Self-assembly. The Structural Hierarchy of Chimeric Drug Delivery Nanosystems
Nikolaos Naziris, PhD student, Pharmacist, MSc
Contributors: Natassa Pippa, Stergios Pispas, Costas Demetzos

17:00 - 17:10
Modern strategies for photothermal and ionizing radiation therapy based on gold nanoparticles-mediated radiosensitization.
Ellas Spyratou, Ph.D., post-Doc Researcher, Department of Radiology, Medical School, NKUA
Contributors: Mersini Makropoulou, Alexandros G .Georgakilas, Efstathios E. Efstathopoulos

17:10 - 17:30
Discussion

Innovative nano-vaccines: From bench to bedside

Natassa Pippa* and Costas Demetzos

Section of Pharmaceutical Technology, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimioupolis Zografou, 15771 Athens, Greece

Abstract

The purpose of this study is to analyze the use of nanosystems in the research and development of innovative vaccines. Nano systems also induce cellular and humoral immunity. A very interesting property of nanosystems is also their adjuvant efficacy, i.e. their ability to enhance the immune response through various mechanisms due to their particulate structure. In immunology, an adjuvant is a component that potentiates the immune responses to an antigen and/or modulates it towards the desired immune responses. It should be emphasized that the nanoparticles themselves contribute to the immune response due to their adjuvant properties, which contributes significantly to the effectiveness of the vaccines.

KEYWORDS: Adjuvants; Innovative Vaccines; Nanoparticles; Immune response

Introduction

Vaccination is one of the most valuable and cost effective health measures to prevent and control the spread of viral/bacterial infectious diseases responsible for high mortality and morbidity.[a,b] A significant number of infectious diseases and chronic disorders is still not preventable by vaccination such as HIV, tuberculosis, malaria, healthcare associated infections (HAIs), cytomegalovirus (CMV), and respiratory syncytial virus (RSV) for which new generation vaccines are needed.1,2Novel technologies such as adjuvants (including immune-modulators and molecular targets) can enable safe and effective vaccines for difficult target populations such as new-borns, elderly and the immune-compromised. The adjuvants need to be very well designed in order to avoid excessive response, long term auto-inflammatory diseases and allergy, secondary effects. Therefore, particulates, and especially liposomes could represent a perfect vaccine adjuvant thanks to the possibility of a high level of customization and control. More recently, liposomes have found application as vaccine –adjuvants due to their ability to prevent antigen degradation and clearance, coupled with enhancing its uptake by professional antigen presenting cells (APCs), have marked liposomes as useful vehicles for the delivery of a diverse vaccine antigen. The majority of vaccines currently in development belong to the category of subunit vaccines, consisting of recombinant or purified pathogen-specific proteins, or encoded (DNA) antigens that will be expressed and presented *in vivo*. Subunit vaccines when administered alone have low efficacy in activating the immune system and require the addition of adjuvants in order to induce a measurable immune response of the antigen, through activation of the innate, and subsequent-

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ly the adaptive immune system. Ideally, the adjuvant should be able to improve antigen uptake by antigen presenting cells (APCs) and induce an antigen specific immune response while eliciting minimal toxicity. Liposomes are a type of adjuvant that can potentially satisfy the above criteria. The adjuvant efficacy of cationic liposomes composed of dimethyldioctadecylammonium bromide and trihalosedibehenate (DDA:TDB) is well established in the literature. Whilst the mechanism behind its immunostimulatory action is not fully understood, the ability of the formulation to promote a "depot effect" is under consideration. The depot effect has been suggested to be primarily due to the cationic nature which results in electrostatic adsorption of the antigen and aggregation of the vehicles at the site of injection.[1,2]

Dendrimers are under investigation as vaccine carriers and/or adjuvants for both infectious diseases and cancer immunotherapy. The adjuvant capacity of mannosylatedPoly(amido)amine (PAMAM) dendrimers was documented in the literature as well as glycopeptides-dendrimers and phosphorus dendrimers. Additionally, polymeric nanoparticles have been applied in vaccine delivery, showing significant adjuvant effects as they can easily be taken up by antigen presenting cells.

The use of nanosystems in the research and development of innovative vaccines

The purpose of this investigation is to analyze the use of nanosystems in the research and development of innovative vaccines. Nano systems also induce cellular and humoral immunity. A very interesting property of nanosystems is also their adjuvant efficacy, i.e. their ability to enhance the immune response through various mechanisms due to their particulate structure. In immunology, an adjuvant is a component that potentiates the immune responses to an antigen and/or modulates it towards the desired immune responses. Currently, two vaccines are available on the market and are based on nano-vesicles. Epaxal®, a hepatitis A vaccine, contains vesicles of approximately 150 nm size consisting of phospholipids (liposomes). The virus particles are adsorbed on virosomes as the adjuvant system composed of highly purified influenza virus surface antigens (10 micrograms haemagglutinin) of the A/Singapore/6/86 (H1N1) strain and the phospholipids lecithin (80 micrograms) and cephalin (20 micrograms). Inflexal V is an influenza vaccine and consists of viral envelope hemagglutinins and lipids. Inflexal V is a vaccine to protect adults and children older than 6 months from flu (influenza). When you are given the vaccine your body's immune system makes antibodies to protect you against the types of flu virus that are in the vaccine. The vaccine does not contain any live virus particles, so it cannot give you the flu. The active substances in the vaccine are proteins called haemagglutinins, isolated from the surface of the flu virus. To make the vaccine, the flu viruses are grown in chicken eggs, then they are inactivated (with ß-propiolactone) and the haemagglutinins are purified. Finally these are combined with natural lipids to form particles called virosomes. The virosomes act as carrier and adjuvant in the vaccine. Additionally, other nanoparticles of lipid and polymeric nature are investigated and developed to be used to deliver antigens and facilitate the vaccine production.

Conclusion

In conclusion, it should be emphasized that the nanoparticles themselves contribute to the immune response due to their adjuvant properties, which contributes significantly to the effectiveness of the vaccines. □

Καινοτόμα νανο-εμβόλια: από την έρευνα στην κλινική εφαρμογή

Νατάσσα Πίππα* και Κώστας Δεμέτζος

Τομέας Φαρμακευτικής Τεχνολογίας, Τμήμα Φαρμακευτικής, Σχολή Επιστημών Υγείας, Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών, Πανεπιστημιούπολη Ζωγράφου 15771, Αθήνα, Ελλάδα
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Περίληψη

Σκοπός της παρούσας εργασίας είναι η ανάλυση της σημασίας των νανοσυστημάτων στην έρευνα και στην ανάπτυξη καινοτόμων εμβολίων. Τα νανοσυστήματα επίσης προκαλούν κυτταρική και χυμική ανοσία. Μία πολύ ενδιαφέρουσα ιδιότητα των νανοσυστημάτων είναι η ανοσοενυσχυτική τους αποτελεσματικότητα, δηλαδή η ικανότητά τους να αυξάνουν την ανοσολογική τους απάντηση με διαφόρους μηχανισμούς εξαιτίας της σωματιδιακής τους φύσης και δομής. Στην ανοσολογία, ένα ανοσοενισχυτικός παράγοντας είναι ένα συστατικό που ενισχύει τις ανοσολογικές αποκρίσεις σε ένα αντιγόνο. Θα πρέπει να υπογραμμιστεί ότι τα ίδια τα νανοσωματίδια συμβάλλουν στην ανοσοαπόκριση λόγω των ανοσοενισχυτικών τους ιδιοτήτων, γεγονός που συμβάλλει σημαντικά στην αποτελεσματικότητα των εμβολίων.

ΛΕΞΕΙΣ-ΚΛΕΙΔΙΑ: Ανοσοενισχυτικός παράγοντας, Καινοτόμα εμβόλια, Νανοσωματίδια, Ανοσολογική απάντηση

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Smart Hybrid Polypeptide-Containing Nanoparticles for the Targeted Delivery of Doxorubicin

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Abstract

The synthesis of novel poly (L-histidine)-grafted mesoporous silica nanoparticles (MSNs) decorated with a uniform pH-sensitive poly(L-histidine) (PHis) shell is reported. Loading and release studies were performed using the model anticancer drug Doxorubicin (DOX). DOX was efficiently loaded within the nanochannels of the hybrid MSN@PHis nanostructures and was released in a relatively controlled pH-triggered manner.

KEYWORDS: Polypeptides; Mesoporous silica nanoparticles; Poly(L-histidine); pH-responsive; Ring-opening polymerization; Grafting from; Controlled drug release

1. Introduction

During the past two decades, there has been tremendous progress in the design of materials at the nanoscale level that has paved the way for a new category of healthcare technologies generally termed nanomedicine. While traditional therapeutic agents have allowed for very little control in terms of their distribution in the body and clearing times, engineering at the nanoscale level has allowed for significant advances in optimizing the biocompatibility, biodistribution, and pharmacokinetics of various medical technologies.

Mesoporous silica nanoparticles (MSNs) have unique characteristics, including high surface area, large specific pore volume, controllable pore diameter and facile surface functionalization[1] as well as their prominent biocompatibility, nontoxicity and high loading capacity apart from their well-ordered structure.[2] However, the weak interactions between physically entrapped drugs or biomolecules and the internal surface of unmodified MSNs, lead to the inevitable burst release of the guest molecules from the nanochannels during the blood circulation.[3] On the contrary, polymer-modified MSNs effectively protect the loaded drugs from premature release and degradation, increasing their therapeutic efficiency.[4]

2. Experimental Section

2.1. Synthesis of Amino-Functionalized MSNs (MSN-NH_2)

Mesoporous silica nanoparticles were prepared by reacting hexadecyltrimethylammonium bromide (CTAB) with NaOH in Milli-Q water at 80°C, followed by addition of tetraethyl orthosilicate under vigorous stirring. The resulting MSNs were isolated by centrifugation and washed thoroughly with Milli-Q water and

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then with methanol, and dried under high-vacuum. The received MSNs were dispersed in methanol by ultrasonication followed by addition of (3-aminopropyl) triethoxysilane. After stirred for 24 hours at room temperature, the product was centrifuged, washed with Milli-Q water and methanol, and then dried *in vacuo*. CTAB was extracted after refluxing the nanoparticles in a mixture of methanol and HCl at 60°C. The amino-functionalized nanoparticles (also referred as MSN-NH2) were isolated by centrifugation and then washed with a saturated solution of LiOH in methanol for the removal of HCl traces from the amino groups.

2.2. Preparation of Poly(L-Histidine)-Grafted MSNs via *Ring-Opening Polymerization (MSN@PHis)*

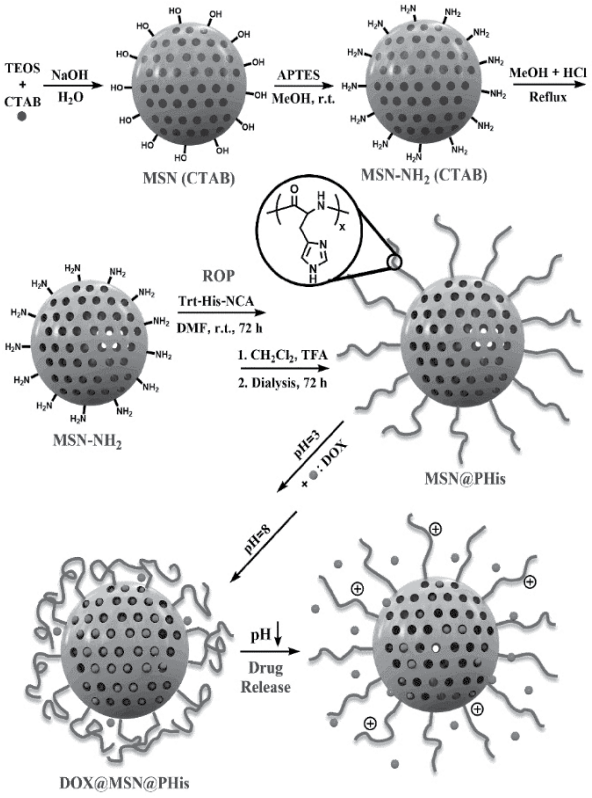
The surface-initiated ring-opening polymerization (ROP) process was carried out by adding the monomer Trt-His-NCA[5,6] to the amino-functionalized mesoporous silica nanoparticles in DMF. The obtained homopolypeptide-grafted nanoparticles solution was then precipitated into excess dry diethyl ether, isolated after filtration and dried under high vacuum (MSN@P(Trt-His), followed by deprotection of the polypeptide with trifluoroacetic acid (TFA).

2.3. DOX Loading and pH-Triggered Release

The prepared amino- and polypeptide-functionalized mesoporous silica nanoparticles were loaded with the model anticancer drug Doxorubicin (DOX), at low pH.

3. Results and Discussion

Scheme 1 shows the functionality of the synthesized nanoparticles.



Scheme 1: *Schematic illustration of the synthetic route followed for the preparation of DOX-loaded PHis-functionalized MSNs for pH-triggered controlled drug release*

4. Conclusions

In this work, we developed novel pH-sensitive poly(L-histidine)-grafted mesoporous silica-based nanoparticles and studied their efficiency as drug carriers of DOX. The DOX entrapment and release pattern were proven to be pH-dependent. □

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In vitro effect of hyperthermic Ag and Au Fe_3O_4 nanoparticles in cancer cell cultures

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Abstract

The use of metal nanoparticles (NPs) in cancer management has gained great attention the last decade. However a lot of research is needed to be conducted regarding the targeting potential, the therapeutic effects, their stability *in vitro* and *in vivo* and their cytotoxicity effects in order to be successfully incorporated in the clinical management of cancer. In the current study, we investigated the cancerous effect of hyperthermic $Fe_3O_{4\ core}$ $Ag(Au)_{\ shell}$ nanoparticles constructed with tryptophan as stabilizer and reducing agent. $Fe_3O_{4\ core}Au_{\ shell}$ NPs seem to have a toxic effect after ionization and thus work as hyperthermic NPs while in the same concentrations the toxic effect of hyperthermic $Fe_3O_{4\ core}Au_{\ shell}$ is minimized in non-cancerous cells HEK293 (~80% viability in all concentrations tested VS ~55% viability in cancer cells with 400μg/ml and 45% viability with 600μg/ml). We suggest that $Fe_3O_{4\ core}Au_{\ shell}$ NPs with Trp can be used as hyperthermic NPs that can selectively have a toxic effect in cancerous cells and not in non-cancerous. However, the role of tryptophan regarding the reduced toxic effects in non-cancerous cells need further investigation.

KEYWORDS: Hyperthermia; Magnetic nanoparticles; $Fe_3O_{4\ core}Ag(Au)_{\ shell}$ nanoparticles; Tryptophan (Trp); anti-tumor effect

Introduction

An increasing number of reviews about noble metal nanoparticles (NPs) in cancer management indicate a growing interest to these promising nanoobjects. Gold and silver NPs can be applied on their own or in combination with other molecules (i.e., polymers, surfactants, organic dyes) for targeting, imaging and therapeutics[1]. In particular, hyperthermic NPs are proven to be a promising tool in cancer therapy. In magnetic hyperthermia, magnetically active NPs like Fe_3O_4 NPs, absorb energy to turn it into heat (>41,5°C). Magnetic NPs have been successfully used in many studies and even progressed in clinical trials[2,3]. However, in order to succeed a specific anti-tumor effect to the tumor site, the construction of targeted NPs seems compulsory. Previously, we estimated the nanotoxicity of colloidal mono- and bimetallic silver/gold NPs stabilized with aminoacid tryptophan (Trp), in three different cell lines 4T, a breast cancer cell line, HCT116, a colon cancer cell line and HEK293, embryonic kidney cell line[4]. Among other results, we found that the toxicity of our NPs was essentially lower in non-cancerouscells, making them promising candidates as tools for cancer treatment approaches[4-6]. Thus, in this study we wanted to check the effect of hyperthermic $Fe_3O_{4\ core}Ag(Au)_{\ shell}$

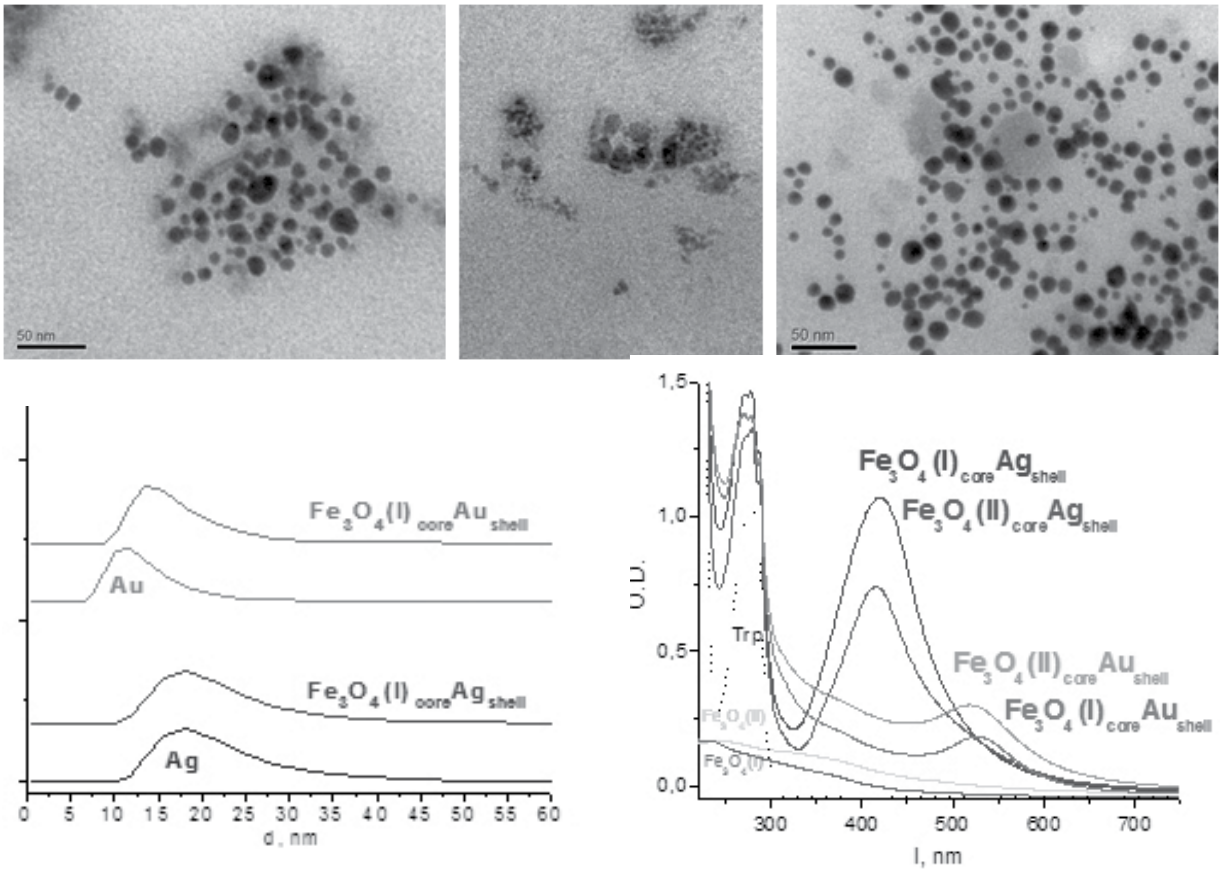

Figure 1: *Properties of Fe3O4 core Ag(Au) shell nanoparticles. Size and morphology of prepared magnetic nanoparticles with the shell of silver (on the left) and gold (on the right). In the middle absorbance spectra of NPs bearing LSPR bands are shown*

nanoparticles nanoparticles in cancer cell cultures[7]. These hyperthermic NPs are stabilized with aminoacid tryptophan as effective in attenuating potential hepatotoxicity and nephrotoxicity of NPs during their *in vivo* application.

Materials and Methods

1. $Fe_3O_{4\ core}$Ag(Au) $_{shell}$ nanoparticles synthesis

Colloidal solutions of nanocomposites containing iron oxide core and noble metal shell $Fe_3O_{4\ core}$ Ag(Au) $_{shell}$ were obtained via chemical reduction of metal ions ($AgNO_3$, $HAuCl_4$, Merck, Germany) by amino acid tryptophan (Trp, SC12-20120713, China) in the presence of magnetic fluid - suspension of iron oxide Fe_3O_4 in sodium oleate. The synthetic procedure was similar to the previously described[4]. Initial solution of Trp was adjusted to high pH and heated to boiling. Then magnetite was injected followed by silver nitrate (tetrachlorauric acid). The components molar ratio was ν(Trp): ν (M): ν (Fe_3O_4) = 2: 1: 0,5. Colloid was stirred and heated continuously.

2. Cell culture

HTC116 and HEK293 cell lines (ATCC) were grown in DMEM High Glucose culture medium (BioSera) containing 10% FBS, 2 mmol/L glutamine, 100 U/ml penicillin and 100 g/ml streptomycin at 37°C. The medium was changed every 48 h and cells were passaged once weekly using standard trypsin-EDTA concentrations. Beginning at passage 32 and 37 respectively,

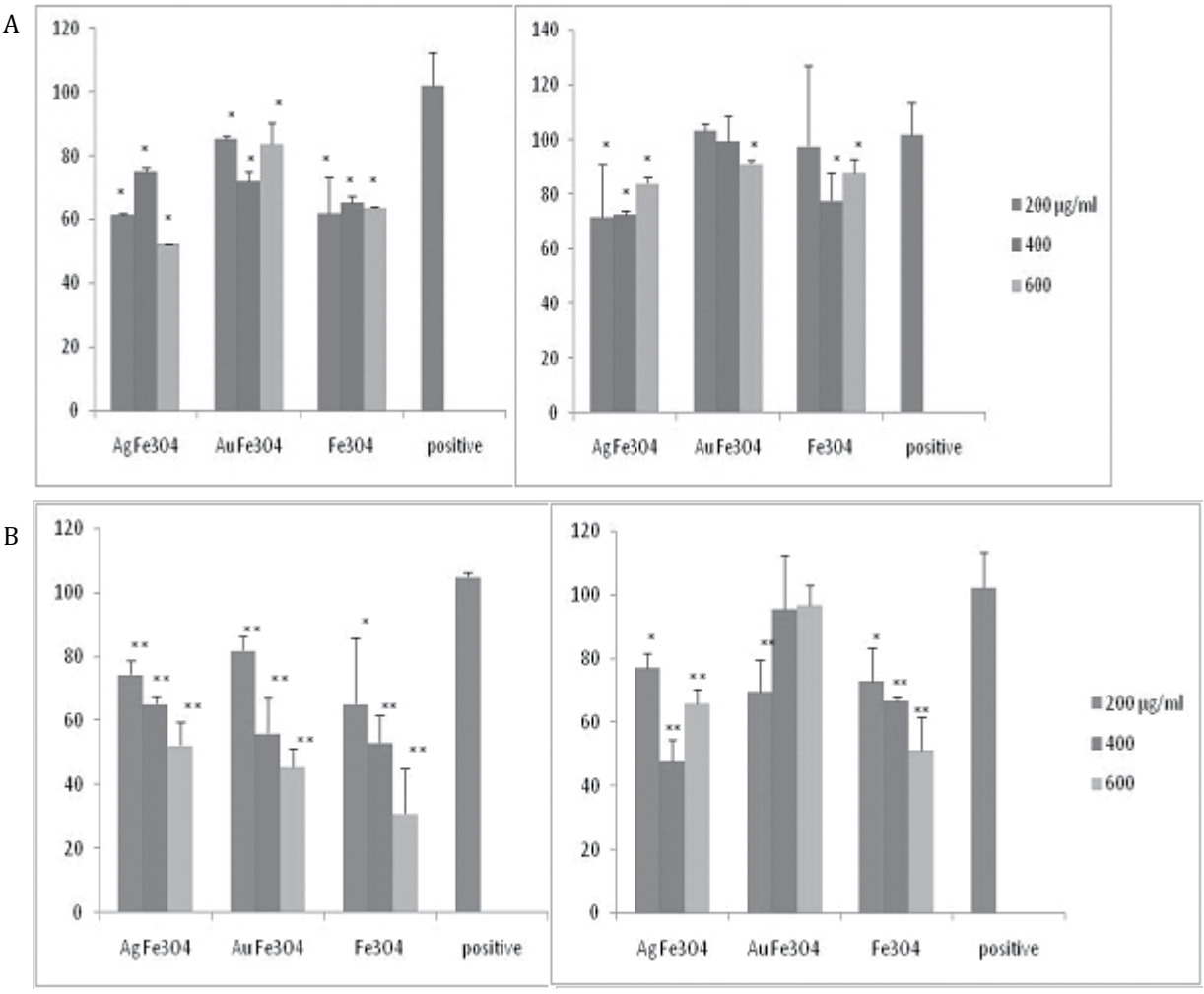

Figure 2: *Viability assay on cell lines. Graph of MTT assay after treatment with various NPs of different concentrations of Fe_3O_4 A) on HEK293 and B) HCT116 and subsequent ionization. Non-ionized cells (graphs on the right) were used as control plates. Positive control shows cells without the exposure of NPs. The cell viability is expressed as % cell viability ±SD between two experiments. The symbols *, **, *** show statistical significance using one way ANOVA (p<0,05, p<0,01, p<0,005 respectively) compared to the positive control*

cells were cultured continuously. Cells were freezed in freezing medium containing FBS, 5% DMSO. HCT116 is a human derived colon cell line. HEK293 are embryonic kidney, epithelial, adherent cells and are used as control group (non-cancerous cell line) in our experiments.

3. MTT assay

The MTT Cell Viability Assay measures alterations in cell viability thus, when metabolic events lead to apoptosis or necrosis, the cell viability is decreased. As a general protocol, 50.000 cells/well were seeded in 24-well plates (Corning-Costar, Corning, NY) and cultured overnight. Three different types of controls, namely: positive, negative and background were used throughout the study. Positive control had cells with culture medium but not exposed to NPs. Negative control had NPs without cells. Background control had culture medium without cells.

The two different cell lines were treated with various concentrations of NPs for 1h and then ionized for 15min. Subsequently, the cells were rinsed once and incubated in 37°C with 100 μl serum-free medium containing 0.5 mg/mL MTT. After 1:30 to 2:30 hours, 100 l of SDS-HCl was added to each well, mixed with the pippete and incubated for at least 1 hour in 37°C. The optical densities were read at 570 nm (reference filter was set at 690 nm), using a microplate spectrophotometer (SPECTROstarNano, BMG LABTECH). Absorbances were normalized with respect to the untreated control cultures to calculate changes in cell viability.

Results and Discussion

Prepared colloidal solutions of magnetic nanoparticles containing iron oxide core and noble metal shell had a bright yellow and red color inherent to NPs of silver and gold respectively.

A specific color is a distinguishing feature of noble metal NPs and is caused by the phenomenon of localized surface plasmon resonance (LSPR) that appears as absorption in the visible range of the spectrum. The maxima of LSPR absorbtion bands of Fe3O4 core Ag shell and Fe3O4 core Au shell colloids were localized at 420 and 527 nm (**Figure 1**), indicating the formation of continuous shell around the magnetite particles. Obtained NPs, with both silver and gold shell, were of spherical shape with the average size of 15-20 nm according to data obtained by transmission electron microscopy (TEM) and dynamic light scattering (DLS) methods (**Figure 1**).

In the present study we also tested the anti-tumor effect of $Fe_3O_{4\ core}Ag_{\ shell}$ and $Fe_3O_{4\ core}Au_{\ shell}$ NPs in a cancerous cell line and a non-cancerous cell line in order to investigate: i) if $Fe_3O_{4\ core}Ag(Au)_{\ shell}$ NPs, reduced and stabilized with Trp, show anti-tumor effect after ionization; ii) if there is a differentiated toxic effect of synthesized NPs on cancerous and non-cancerous cells.

According to our results, the NPs that have a toxic effect towards cancer cell after ionization, and thus work as hyperthermic NPs, are $Fe_3O_{4\ core}Au_{\ shell}$ NPs in the concentrations 400 and 600 μg/ml of Fe_3O_4 (**Figure 2B**). $Fe_3O_{4\ core}Ag_{\ shell}$ NPs because of the toxic effect of AgNPs on their own, compared to AuNPs that are considered to be the less toxic metal NPs for *in vivo* applications[4,8]. Interestingly, in the same concentrations the toxic effect of hyperthermic $Fe_3O_{4\ core}Au_{\ shell}$ NPs is minimized in non-cancerous cells HEK293 (**Figure 2A**) (~80% viability in all concentrations tested VS ~55% viability in cancer cells with 400μg/ml and 45% viability with 600μg/ml) (**Figure 2A and B**). We deemed that this specificity towards cancer cells has to do with the use of tryptophan as a stabilizer and reducing agent. Tryptophan is demonstrated to have rather a positive effect on both cell lines compared to the positive control, especially on cancer cells (data not shown). Thus, the increased metabolism of Trp by cancer cells specifically, may be beneficial in order to increase the anti-tumor effect of NPs[9]. Therefore, we suggest that $Fe_3O_{4\ core}Au_{\ shell}$ NPs with Trp can be used as hyperthermic NPs that can selectively have a toxic effect in cancerous cells and not in non-cancerous without the need of conjugation in order to target cancer cells. □

In vitro δράση υπέρθερμικών Fe_3O_4 νάνοσωματιδίων Ag και Au σε καλλιέργειες καρκινικών κυττάρων

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Περίληψη

Η χρήση μεταλλικών νανοσωματιδίων (NPs) στη διαχείριση του καρκίνου έχει κερδίσει μεγάλο ενδιαφέρον την τελευταία δεκαετία. Ωστόσο, πρέπει να διεξαχθούν πολλές έρευνες σχετικά με την ειδική στόχευση των καρκινι-

κών κυττάρων, τα θεραπευτικά αποτελέσματα, τη σταθερότητά τους *in vitro* και *in vivo* και την κυτταροτοξικότητά τους προκειμένου να ενσωματωθούν με επιτυχία στην κλινική αντιμετώπιση του καρκίνου. Στην παρούσα μελέτη, θελήσαμε να ερευνήσουμε την καρκινική επίδραση των υπερθερμικών νανοσωματιδίων Ag και Au Fe_3O_4 κατασκευασμένων με τρυπτοφάνη ως σταθεροποιητή και αναγωγικό παράγοντα. Τα NPs Fe_3O_4 (πυρήνα) Au (κέλυφος) φαίνεται να έχουν τοξική δράση μετά από ακτινοβόληση και άρα να λειτουργούν ως υπερθερμικά NPs. Παράλληλα, στις ίδιες συγκεντρώσεις το τοξικό αποτέλεσμα των υπερθερμικών Au Fe_3O_4 NPs ελαχιστοποιείται στα μη καρκινικά κύτταρα HEK293 (~ 80% βιωσιμότητα σε όλες τις συγκεντρώσεις που εξετάστηκαν VS ~ 55% βιωσιμότητα σε καρκινικά κύτταρα με 400 μg / ml και 45% βιωσιμότητα με 600 μg / ml). Εν κατακλείδι, προτείνεται πως τα NPs Fe_3O_4 (πυρήνα) Au (κέλυφος) με Trp μπορούν να χρησιμοποιηθούν ως υπερθερμικά NPs που μπορούν επιλεκτικά να έχουν τοξική επίδραση σε καρκινικά κύτταρα και όχι σε μη καρκινικά κύτταρα. Ωστόσο, ο ρόλος της τρυπτοφάνης όσον αφορά τις μειωμένες τοξικές επιδράσεις σε μη καρκινικά κύτταρα χρειάζεται περαιτέρω διερεύνηση.

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Folate-positive cancer cell biosensor based on an electroactive hydrogel doped with silver enhanced/folic acid functionalized gold nanoparticle-targeted cells

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Abstract

In the present work we report an electrochemical approach for assaying folate targeting in cancer cells. The proposed assembly is based on coulometric evaluation of silver enhanced MCF-7 cells after folic acid functionalized gold nanoparticle targeting. Total charge of an electroactive hydrogel doped with nanoparticle targeted-silver enhanced cells was used as a figure of merit. The assembly was able to measure the total charge of the enhanced hydrogel, differentiate between unmodified/modified nanoparticle targeting and between different modified nanoparticle concentrations.

1. Introduction

A biosensor is characterized by its biological recognition element which bears unique specificities toward corresponding analytes. One of the many challenges in biosensor development is the efficient signal capture of the recognition event (transduction). Transducers translate the interaction of the biological element with the analyte into an electrochemical, magnetic, gravimetric, luminescent or optical response. In the quest of increased biosensor sensitivities, nanomaterials are promising candidates because they offer enhanced quantity of immobilized bioreceptor units at reduced volumes, while they often can act themselves as the transduction element[1]. Continuous advances in synthesis and functionalization of gold nanoparticles (AuNPs), combined with their biocompatibility, chemo-physical stability and optical tunable characteristics, have led to an immense expansion of their biosensory applications. Folic acid (FA) mediated cancer cell targeting, which is based on the overexpression of FA receptors by certain cancer cells, is one of the most important methods for active cancer targeting and diagnostics[2]. The aim of the present study was to develop a biosensor assembly that will electrochemically assess folate-conjugated gold nanoparticle targeting of cancer cells.

2. Materials and Methods

2.1 Materials

Silver nitrate (AgNO3), KNO3, hydroquinone were obtained from Merck (Darmstadt, Germany). Glutathione (GSH) and MES were purchased from Fluka (Basel, Switzerland). All other reagents were purchased from Sigma-Aldrich.

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2.2 Preparation of folic acid functionalized gold nanoparticles

Prior to experiments, all glassware were cleaned with aqua regia (3:1 HCl:HNO_3). For the colloidal synthesis, gold solution was prepared by dissolving $HAuCl_4$ (14 mg) in ddH_2O (116.6 mL). A freshly prepared $NaBH_4$ solution (2.5 mg / mL, 466 μL) was added dropwise to $HAuCl_4$ and the solution was stirred for 5 min until turned ruby red in color. Afterwards, GSH (93 mg) was added and the colloidal solution was stirred for 15 min. Then, the mixture was centrifuged at 4,500 rpm for 10 min and the precipitant was collected. Consequently GSH capped-AuNPs (58 mg) were dispersed in 29 ml MES buffer (50 mM, pH 6.5) with folic acid (29 mg), sulfo-NHS (14.5 mg) and EDC (26.1 mg). After 24h under vigorous mixing, the particles were separated by adding 15 ml of methanol and centrifuging at 4.500 rpm for 10 min. The procedure was repeated three times and the gold nanoparticles were allowed to dry on a watch glass for 24h[3].

2.3 Cell culture and fixation

MCF-7 (kindly provided by Dr. Bouziotis and Dr. Paravatou from N.C.S.R "Demokritos") cells were grown and maintained in 75 cm2 culture flasks (OrFlask, Orange Scientific) with DMEM medium enriched with 10% FBS, 1% L-glutamine, antibiotic solution and 1% pyruvic acid at 37°C / 5% CO_2. For microscopic observations cells were plated in 8 well slides (Lab-Tek® II Chamber Slide ™ System, 1545348) at a concentration of 4 x 104 cells/cm2. After cell adhesion (24h) the medium was removed and cells were incubated for 1h with DMEM without FBS containing the FA-AuNPs at 10 mg/ml and 50 mg/ml respectively. At the end of incubation time, cells were fixed for 20 min at 4°C with glutaraldehyde (Glu).

2.4 Fixation and silver enhancement for microscopy

Silver enhancement solution was prepared by dissolving 0.51 g of citric acid and 0.48 g of trisodium citrate in 20 ml of distilled water (4° C). Then, 0.02 g of hydroquinone was added under darkroom conditions. The pH was adjusted to 3.8-4.0 with citric acid and 0.07 g $AgNO_3$ was added to the solution under stirring (10 min). The solution was used quickly after preparation. The plate was rinsed 3 times with ddH_2O.

2.5 Cell fixation and silver enhancement for electrochemical measurements

After MCF7 scraping, cells were centrifuged at 1500 rpm for 5 minutes, followed by 2 washes in sterile PBS and fixation with Glu (1%). All samples were incubated for 20 min and centrifugated with sterile PBS for 7 min at 3000 rpm. The samples were left to dry at RT before silver-enhanced as above.

2.6 Preparation of gelatin hydrogel and immobilization on gold electrodes

For hydrogel formation, 10% gelatin was dissolved in deionized water and heated for 30 minutes at 55° C. After that, 5 μl of the silver enhanced cells and 5 μl of the gelatin were mixed to a final concentration of 5% and deposited (drop-casting) on the working electrode of the 220AT gold screen printed electrodes (Dropsens, Spain). The electrodes were left to dry until solidification of the films.

2.7 Electrochemical measurements

Prior to connecting the electrode to the potentiostat, 70 μl of potassium nitrate (KNO3) was added to the electrode. Chronocoulometry was performed using UiEChem™ software from Bio-Logic Science Instruments SAS (Claix, France). Measurement conditions were: applied potential +0.8V and sample rate 5Hz, cumulative Charge vs. Time. Total charge was calculated automatically from the pre-installed UiEChem™ software application[4].

3. Results and Discussion

Both AuNPs and AuNPs-FA exhibited a peak around 526nm (**Figure 1A**) that corresponds to

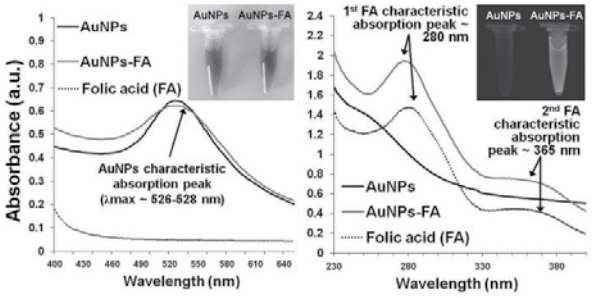

Figure 1: *UV-Vis absorption spectra of AuNPs and FA functionalized AuNPs. Inserts: corresponding optical and UV illuminated images*

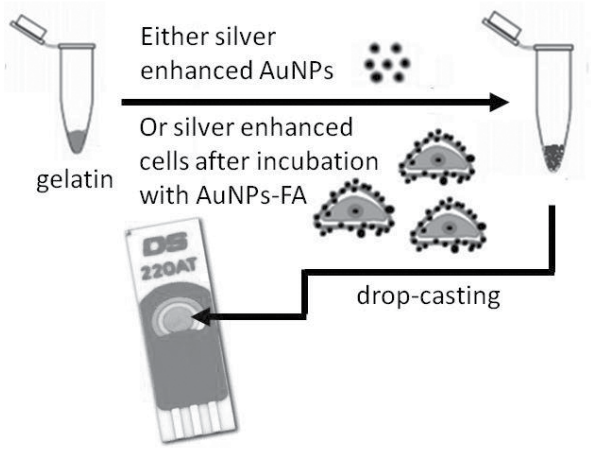

Figure 3: *Schematic representation of the measurement setup*

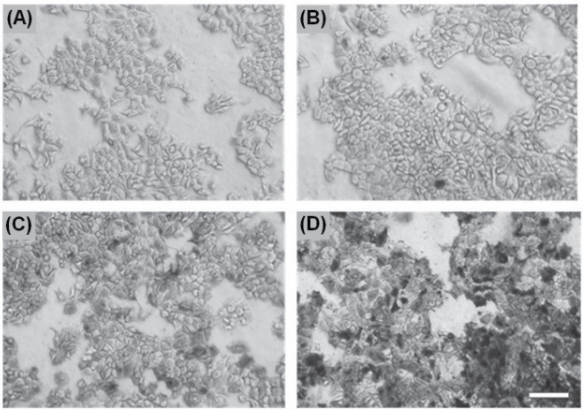

Figure 2: *Targeting of AuNPs-FA in MCF7 cells via silver enhancement. A) control, B) unmodified AuNPs, C) AuNPs-FA 10μg/ml and D) AuNPs-FA 50μg/ml*

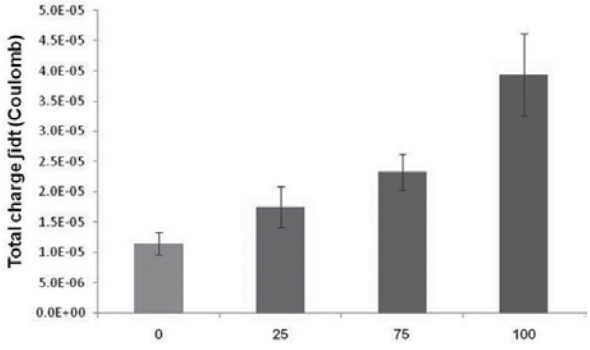

Figure 4: *Total charge of electrode/ hydrogel/AuNps configurations*

the characteristic SPR band of AuNPs in the visible region, suggesting the creation of Au nanoparticles.

In the UV spectra (**Figure 1B**), the absorption max of AuNPs-FA at 280 nm and 365 nm, both characteristic peaks of folic acid, confirmed their covalent attachment with FA.

In order to evaluate NP/cell interactions with optical microscopy, the silver enhancement method was performed (**Figure 2**). Cells incubated with no or unmodified AuNPs (**Figure 2 A,B**) showed less stain intensity than cells with FA-AuNPs. Additionally, microscopy of cells treated with FA-AuNPs revealed dose depended staining (**Figure 2 C, D**).

The electrochemical assembly presented in this work was based on the immobilization of AuNPs or AuNPs-targeted cells on a gold electrode through a hydrogel film formation as presented in Fig.3. Chronocoulometry results showed that the total charge transferred from the electrode/hydrogel interface for the case of immobilized AuNPs was dose dependent (**Figure 4**). As the increased AuNPs concentrations resulted in increased silver enhancement, the silver deposits allowed higher current transfer from the electrode and higher total charge in a dose response manner.

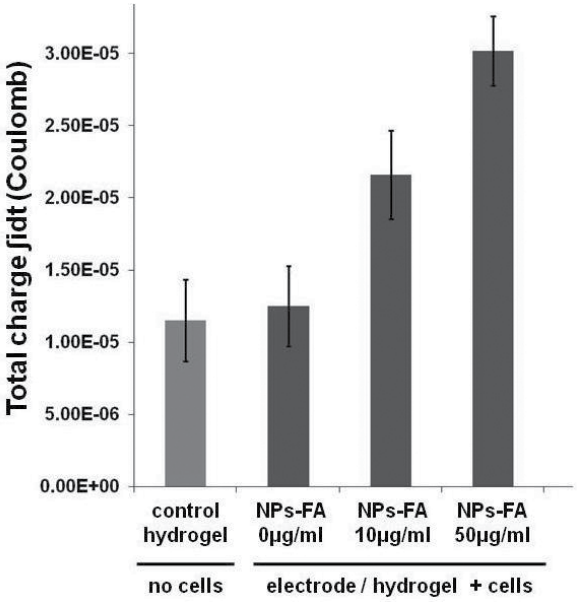

Figure 5: *Total charge of electrode/ hydrogel/cells/ FA-AuNPs configurations*

In the case of immobilized AuNPs-targeted cells the total charge of the electrode/hydrogel interface for the cells incubated with no NPs was similar to the control (no cells). Additionally, total charge in hydrogel/cells configuration increased as the concentration of the FA-AuNPs increased (**Figure 5**). Results revealed that incubation of cells with FA-AuNPs showed targeting both microscopically and electrochemically.

4. Conclusions

The proposed bioelectrochemical assembly was able to measure the charge of gold nanoparticle folate-targeted cells and discriminate between unmodified AuNPs or between different FA-AuNP concentrations.

Further studies are needed to evaluate tissue specific selectivity and minimum cell populations required for signal acquisition. □

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Nanomedicine in diagnosis and treatment of neurological diseases. A literature review

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Abstract

The reported increasing burden of neurological diseases over the last years highlights the need to identify new approaches in diagnosis and management of neurological diseases. Nowadays, nanomedicine provides new powerful tools for the imaging and treatment of various neurological diseases. In neuroimaging, nanoparticles have been successfully used as contrast agents to enhance visualization in Magnetic Resonance Imaging (MRI), Computed Tomography (CT) and photoacoustic imaging. The ability of nanoparticless to effectively cross the Blood Brain Barrier (BBB) make them ideal candidates for targeted dug delivery. Consequently, nanoparticles seem to offer new perspectives for a more accurate diagnosis and effective treatment in the field of neurology. The aim of the current review study is present the main applications of nanoparticles in diagnosis and treatment of neurological diseases.

1. Introduction

Neurological diseases including cerebral aneurysms, inflammation, multiple sclerosis, Alzheimer, Parkinson and brain tumors are an important cause of disability and deaths worldwide. According to the Global Burden of Diseases 2015, neurological diseases were the leading cause of global disability-adjusted life years (DALYs) and the second-leading cause of deaths in 2015[1]. Therefore, there is an urgent need to find new approaches for the management of neurological diseases.

In recent years the development of nanotechnology has offered new and significant opportunities in diagnosis and treatment of neurological diseases[2]. Nanoparticles are a novel tool in the management of neurological diseases as they offer a number of advantages, such as: small size (1-200nm), high stability, biocompatibility and the ability to selectively bind with specific biological molecules and act as drug carriers[3].

The aim of the current review study is to present the main applications and the role of nanomedicine in diagnosis and treatment of neurological diseases. More specific, the article is focused on the use of nanoparticles as contrast agents in Magnetic Resonance Imaging (MRI), CT (Computed Tomography) and photoacoustic imaging as well as on the use of nanoparticles as drug carriers in neurology.

2. Materials and Methods

A thorough literature search was conducted using the database PubMed for studies published the

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last decade with the following keywords: [nanomedicine or nanotechnology or nanoparticles or nano-contrast agents or nanocarriers] and [neurology or central nervous system diseases (CNS)].

3. Results, Discussion, Conclusion

In the field of neuroimaging, the literature search revealed that nanoparticles have been successfully used as contrast agents in clinical MRI studies and in preclinical CT and photoacoustic applications. The most popular nano-contrast agents for MRI are iron oxide nanoparticles (IONs). IONs display ideal properties required for MRI contrast agents as they have a prolonged blood residence time and can be used even in patients with chronic renal failure[4,5]. A clinical study conducted by Tourdias et al.[6] for the evaluation of disease activity in multiple sclerosis showed that IONs-enhanced MRI help to identify additional lesions compared to gadolinium contrast agent. Moreover, relevant studies demonstrated that IONs improve the visualization of amyloid plaque and CNS inflammations and enhance the contrast in brain tumors[2]. Concerning CT and photoacoustic imaging, gold nanoparticles (AuNPs) are the most commonly used nano-contrast agents. Kim et al. revealed that Fibrin-target AuNs allow the prompt detection and quantification of cerebral thrombi using micro CT[7] whereas photoacoustic imaging in combination with PEG-AuNs improves the visualization of brain vasculature structure[8]. As presented in recent literature, nano-contrast agents in MRI, CT and photoacoustic imaging enables detailed diagnosis of neurological diseases at molecular and cellular level therefore leading to a significant improvement in the diagnostic accuracy of the aforementioned techniques[4,9].

In the treatment of neurological diseases, the most challenge issue is drug transport through the blood-brain barrier (BBB). According to several studies, various types of nanoparticles can be used as nanocarriers for target drug delivery, especially after being activated by specific ligands[10,11]. Due to their small size, nanoparticles cross the BBB and seem to permit a more effective and target-oriented treatment compared to traditional drug delivery systems. Liu et al. showed that B6 peptide-modified PEG-PLA nanoparticles enhance drug uptake across the BBB for the treatment of Alzheimer disease[12]. In a research study for the treatment of Parkinson's in rats, Pahuja et al., found that dopamine-loaded PGLA nanoparticles increase dopamine level and reduce dopamine-D2 supersensibility[13]. Additional, a similar study, demonstrated that the use of poly(butyl cyanoacrylate) nanoparticles provides significant reduction of the basic symptoms of Parkinson (oligokinesia, rigidity, tremor)[14].

Nanoparticles seem to be promising agents for the management of several neurological diseases while at the same time their use is getting significant attention in the field of theranostics[15]. However, a detailed understanding of the nanotoxicity mechanisms and ethical issues still remain a challenge for the establishment of nanoparticles in clinical neurology. □

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Developing therapeutic drug delivery nanosystems from different lyotropic liquid crystalline mesophases

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Extended Abstract

The use of lyotropic liquid crystals is an innovative approach towards the development of therapeutic nanosystems delivering agents, such as drugs, proteins and nucleic acids. Lyotropic liquid crystals are formed via the dispersion of amphiphilic molecules to the water. Regarding of their water concentration and the temperature, amphiphilic molecules are self-assembled to different mesophases, such as micelles, lamellar, hexagonal and cubic structures (**Figure 1**), while the hydrophobic and lyotropic effects affect the formation of lyotropic liquid crystals in a hierarchical manner. Thus the curvature of the structures is driven by the concentration of the biomaterials used for the formation of the liquid crystalline state of matter. As a result, lyotropic liquid crystals present great structural variety and internal organization, providing different kinds of applications at the field of therapeutic nanotechnology.

All above mesophases can be exploited to develop different kinds of vehicles that are all categorized at liquid crystalline nanosystems (**Figure 1**). Cubosomes and hexosomes are classified at the non-lamellar ones and are of the most promising drug delivery nanosystems. They present many advantages, including nanostructural versatility, compatibility, digestibility, tunable morphological characteristics, as well as the capability of solubilizing and sustaining the release of amphiphilic, hydrophobic and hydrophilic agents[1,2].

More specifically, the cubic phases are classified into two different nanostructures of either bicontinuous

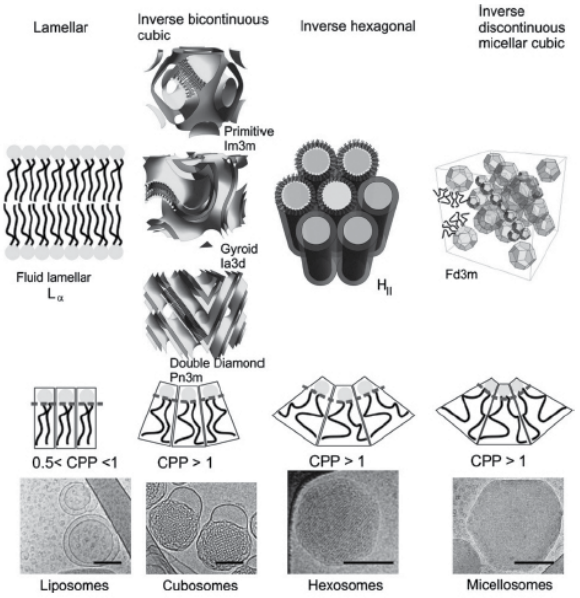

Figure 1: *Structural configuration of self-assembled systems and corresponding cryo-transmission electron microscopy images showing morphology of dispersed nanostructured particles. (Adapted from X. Mulet et al., Journal of Colloid and Interface Science 2013;393:1–20)*

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(V2) or discontinuous (I2) water compartments. The bicontinuous cubic (V2) phase is a complex structure, consisted of a 3D network, separating two distinct, continuous but nonintersecting, hydrophilic sections. Three types of bicontinuous cubic phases with different space groups have been identified in various lipid systems, named as Im3m (primitive type), Pn3m (double-diamond type) and Ia3d (gyroid type) assemblies. The discontinuous cubic (I2) phase of the symmetry Fd3m consists of two different quasispherical close-packed micelles in a 3D cubic lattice. Regarding of the inverse hexagonal (H2) phase, it is a 2D structure consisting of water-filled cylindrical rods (hydrophilic nanochannels), embedded in a continuous hydrophobic medium.

Glyceryl monooleate (GMO, 2,3-dihydroxypropyl oleate), phytantriol (PT, 3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) and other lipids, such as phospholipids, are proved to form cubic and hexagonal phase under specificate conditions of water concentration and temperature. Followed by the addition of stabilizers, such as Pluronic copolymers, stable colloidal dispersions, cubosomes and hexosomes respectively, are formed. There are two commonly used preparation methods, being referred at the literature, named top-down and bottom-up techniques[1-3].

The scope of our study is to investigate the optimal liquid crystalline nanosystem formulation, depending on its future loaded agent and its future target. Different combinations of functional biomaterials (lipids or stabilizers) will upgrade the conventional formulations of liquid crystalline nanosystems to advanced nanosystems.

The physicochemical (size, size distribution and ζ-potential), morphological and thermotropic characteristics of the prepared nanosystems should be evaluated. There is a variety of imaging techniques that can be used to characterize the morphology, as well as the internal structure. While lyotropic liquid crystals undergo structural polymorphism, the influence of the environmental conditions, such as temperature, should also be studied and their biophysical behavior be highlighted. Subsequently to the above characterization process, when therapeutic agents are loaded to the liquid crystalline nanosystems, the carrier should also be re-characterized, in order to confirm any possible alterations being caused by the loaded agent[1-4].

In conclusion, non-lamellar liquid crystalline nanosystems are considered to be a well promising category of therapeutic nanosystems that can be transformed to advanced drug delivery nanosystems by the proper biomaterial modification. □

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(*) The above study is carried out during the PhD studies, under the supervision of Costas Demetzos (Professor, Department of Pharmacy, National and Kapodistrian University of Athens), Stergios Pispas (Director of Research, Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation) and Nektarios Tavernarakis (Chairman of Foundation for Research and Technology –Hellas, Director of Research, Institute of Molecular Biology and Biotechnology, Professor, Medical School, University of Crete).

Physicochemical characteristics of liposomes and their lyotropism influence for protein-liposome interactions *in vitro*

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Extended Abstract

Liposomes are defined as nanosized vesicles composed of amphiphilic molecules that are able to self-assemble in water forming lipid bilayers. Liposomes can consist of one or more lipid bilayers, where the polar head groups are exposed to water, interacting with the aqueous environment. They can incorporate both hydrophilic and lipophilic bioactive molecules, which are entrapped in their aqueous core or added to the bilayer. Liposomal nanomedicines can be used in the treatment of many diseases, like cancer, since liposome encapsulation of drugs can reduce toxicity and increase the efficacy.[1] However, one of the main problems of their application is that when they enter in the bloodstream, they are recognized from the macrophage-phagocyte system and as a result they are opsonized leading to side effects.[2]

Moreover, when proteins bound to the liposomes, they alter the physicochemical properties of liposomes, such as size and surface charge and consequently biodistribution and clearance are also affected.

Thus, the aim of the present study is to investigate the effects of serum proteins on the physicochemical characteristics of liposomes as well as the role of lipid composition and concentration in the interactions between serum proteins and liposomes. In this study, different liposomal formulations were made using neutral and charged phospholipids, cholesterol and polyethylene glycol (PEG)-lipid conjugates. Multilamellar vesicles (MLVs) were produced by the thin-film hydration method and they were sonicated resulting in the production of Small unilamellar liposomes (SUVs). To determine their physical stability, liposomes were dispersed in HPLC-grade water and their size, size distribution and zeta-potential were measured for a time period of 28 days with dynamic and electrophoretic light scattering.

The same physicochemical parameters were measured for liposomes after they were dispersed in Fetal bovine serum in order to see how Bovine serum albumin interacts with liposomes with different ways (i.e. electrostatically, etc.). Our results show that liposomes formulated only by phospholipids were not thermodynamically stable over time and aggregation phenomena induced by serum proteins was observed. On the contrary, negatively charged liposomes containing cholesterol and PEGylated liposomes exhibited stealth properties preventing protein binding due to the electrostatic and steric repulsive forces. In addition, the incorporation of cholesterol to all above liposomes confer stability due to the cholesterol-induced formation of ordered membranes.[3]

In this paper, we propose a new factor named Fraction of stealthness (Fs) for evaluating the extent of protein binding and how concentration changes affect the protein adsorption. Fraction of stealthness is based on size changes of liposomes before and after incubation in serum and it can have values between 0, extensive protein binding, and approximately 1, stealth liposomes. The negative charged and the polyethylene glycol coated li-

posomes have stealth properties since our results showed that Fs is close to 1.

Finally, the slopes (a) of equations of Fs versus lipid concentration indicate that there is a concentration dependence of protein binding. When the slope is negative more proteins are adsorbed at highest concentration, whereas when the slope is positive the opposite phenomenon is observed. □

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The Role of the Information/ Entropy Balance in Self-assembly. The Structural Hierarchy of Chimeric Drug Delivery Nanosystems

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Abstract

Chimeric/mixed nanosystems are the next step on the road to develop "smart" medicines, by integrating different classes of biomaterials to overcome drug delivery limitations. In the present study, an approach on the formation and functionality of chimeric nanosystems is suggested, which is based on the information/entropy relationship. The combination of nanomaterials leads to system complexity and consequently, alters the energetic pathway followed during self-assembly, according to the properties and contained information of these molecules. Intermediate phases (i.e. metastable) seem also to play a role in the final morphologies to be obtained. Such approaches might contribute to our understanding the mechanisms of nature, while leading us one step closer to producing more effective and safe drug delivery systems.

KEYWORDS: Chimeric nanosystems; Biomaterials; Self-assembly; Information/entropy

Introduction

Pharmaceutical Nanotechnology is a multidisciplinary field that combines knowledge from several other scientific fields to deliver efficient and safe therapeutic applications. Among the latter, one of the most promising is drug delivery nanosystems (DDnSs), including e.g. liposomes and polymersomes. In addition, chimeric/mixed nanosystems, which combine more than one class of biomaterials e.g. lipids and polymers, are promising candidates for achieving targeted delivery of drugs, bypassing the hurdles generated by the physiological complexity that conventional ones fail to achieve[1]. First-generation DDnSs and especially liposomes, which have long been on the market, were of the simplest composition, most containing a single class of biomaterials e.g. one or two phospholipids, cholesterol and PEG-lipids[2]. This has served so far concerning development control and easy scale-

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up of these formulations, which are prerequisites for pre-clinical and clinical success. The various phospholipids utilized for liposome preparation are similar in chemical structure and properties and for this reason they behave similarly in terms of self-assembly and match with each other. However, these products are not considered as "smart" or "intelligent" and their fate is determined by the physiological complexity and diversity. In order to potentiate completely guided drug delivery, we need to move on to more sophisticated "recipes", such as chimeric advanced delivery nanosystems (chi-aDDnSs), composed of more than one class of biomaterials, which brings us closer to the complex composition of biological systems[3,4].

Self-assembly

Concerning self-assembly, it is a term that describes spontaneous processes, wherein disorganized systems of molecules or other nanoentities are driven to organized structures or patterns, via their local physical interactions with each other and final minimization of the system free energy, by minimizing repulsive and maximizing attractive forces. This irregular-to-regular arrangements transition is primarily enthalpy-driven, but can in some cases be entropy-driven. Self-assembly is explained by statistical thermodynamics and in particular by the second law of thermodynamics and the Gibbs free energy equation, which predicts if a given process is going to happen spontaneously or not:

$$\Delta G_{SA} = \Delta H_{SA} - T\Delta S_{SA} \quad (1)$$

If the free energy transition ΔGSA, where SA stands for self-assembly is negative, then the process will occur spontaneously. In order for that to happen, the transition enthalpy will have to be sufficiently negative, compared with the transition entropy. The first is determined the intermolecular forces and potential energy between the assembling molecules, while the second represents the ordered/hierarchical arrangement of structures. Generally, entropy must decrease for the self-assembly to be driven, however this transition varies amongst different systems of biomaterials. Finally, in such systems, conservation of energy and the first law of thermodynamics apply only if someone considers the whole thermodynamic system e.g. colloidal. However, inside that, interactions, energy and even mass exchanges between the individual structures continue to occur after thermodynamic stability has been achieved. For examples, the formation of thermodynamically stable aggregates from thermodynamically less stable molecules or supramolecular complexes (e.g. liposomes) is of high hierarchical selection[5].

Biomaterials

Synthetic biomembranes are simple, making their development control easy to handle. However physiological membranes are way more complex, containing many classes of biomaterials e.g. lipids, polysaccharides and peptides, which somehow nature has managed to organize during the first steps of life[4,6]. The higher the system complexity, the higher the number of informational pathways that interact to produce a specific information-entropy balance of the system. However, when attempting to achieve the production of complex membranes on bench, we end up in total failure, because of a plethora of reasons, associated for example with molecular dynamics, metastable phases and phase separation in such systems[7].

Molecules contain certain information, which is defined by properties like their chemical structure, conformation, molecular weight etc. When mixing different biomaterials, the deviation of informational content between them means mismatch of their physical properties and resulting surfaces, which defines their intermixing and final self-assembly into structures, through a thermodynamic process. This informational gap then defines the transition enthalpy, related with the intermolecular interactions, as well as the transition entropy, associated with the degree of order of arranged structures[5]. Hence, the system of self-assembled structures where free energy will be minimum is directly linked with the initial information, which defines the final energetic content and degree of

order of the system, which in turn compose its energy-entropy or information-entropy balance.
A part of our laboratory research, regards the parameters that govern the self-assembly process in nanocolloidal systems and especially chimeric liposomes. A study utilizing cryo-transmission electron microscopy (cryo-TEM) highlighted/pointed out the role of the biomaterial e.g. block copolymer properties on the lyotropism, morphogenesis of new nanomorphologies and as a result, on the biological stability and effectiveness of these systems[8]. Moreover, biophysics and thermodynamics are used in our research in order to expand our knowledge on the behavior of complex nanosystems that are able to deliver drug into pathological tissues[9]. Herein, we explain how the information/entropy balance is defined in a chimeric nanosystem, how it affects the thermodynamic process of self-assembly of different in nature biomaterials into various structures, what its physical meaning is and what the biophysical outcomes of this relationship are.

Energy and Information

In 1956, Léon Nicolas Brillouin came up with the formula[10]:

$$K_B ln2(J/K) \ (2)$$

This represents the amount of entropy the system must decrease by to obtain 1bit of information, where KB = 1.38 × 10-23 is the Boltzmann constant.

In 1948, Claude Shannon defined the entropy H (from Boltzmann's H-theorem) of a discrete random variable X with possible values {x1, x2 ..., xn} and probability mass function p(x) as[11]:

$$H(X) = -\sum_{i=1}^{n} p(x_i) log_b p(x_i) \ (3)$$

Where b is the base of the logarithm used and the common value of b is 2. The theory solved the problem of quantitative metrics in information.

Finally, in 2013, Chan and Jian, based on Ludwig Boltzmann, proposed that the link between information and entropy can be given as[12]:

$$\sum I \leftrightarrow \sum E \ (4)$$

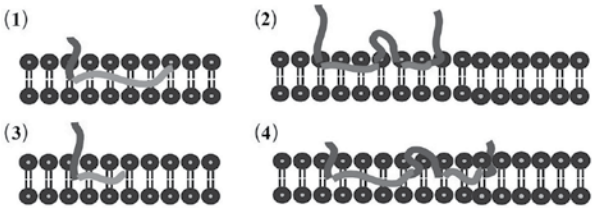

Figure 1: *Lyotropism affects the biophysics of chimeric drug delivery nanosystems by promoting structural polymorphism, due to the incorporation of the polymeric guest into the lipid bilayers of the nanosystem. The complexity of chimeric drug delivery nanosystems arises from the combination of different in nature biomaterials, i.e. phospholipids and polymers, resulting in better functionality and effectiveness of advanced drug delivery nanoplatforms [Adapted from ref. 16]*

In their effort to develop sustainable energy system, laying down the general guidelines for the integration of Energy and Information.

The Information/Entropy Balance in Chimeric Drug Delivery Nanosystems

Entropy and information are directly related terms according to the Shannon published work in 1948 on the *Mathematical Theory of Communication*[11]. Information theory is connected to the entropy and functionality of chimeric nanostructures. Biomaterial information and relative molar balance profoundly affect the statistics of the organization process and as a result, the hierarchical selection of formed biostructures, where intermediate phases of structural polymorphism can be found. Any changes in entropy, which means changes in the heat content of the nanosystem, could be considered as the driving force for altering the micro-organization process of the morphology and the structural dynamics and asymmetry of the nanosystem and consequently, its macroscopic structure.

We can disorganize a system by applying heat or by withdrawing information. Information/entropy balance could serve as a tool to tune the development of nanosystems, where the self-assembly of such biomembranes mimics mechanisms occurring in nature.

In the case of lyotropic liquid crystals the concentration could be considered as the "structural composer" that adds or remove heat by using the hydrophobic forces as the main instrument, affects the micro-organization of the nanosystem and produces different morphologies. Because information has similar behavior as energy, it is involved in the self-assembly process of nanosystems and promotes different in morphology structures, in a well-defined hierarchical morphological cascade.

The lyotropism of biomaterials, mainly concentration, defines the self-assembly of structures, wherein information and entropy are hidden elements and their balance affects the biophysics of nanocarriers and as a result, their final biological effectiveness (**Figure 1**).

The energy-entropy balance, which is related to the information-entropy balance, is present in the self-assembly process and depending on where it leans, different supramolecular structures are produced, based on the hierarchical selection of structures, as defined by the system. The balance is also present in the final structures and in their colloidal system generally, where the free energy minimum has been achieved and thermodynamic balance/steady state exists, according to statistical thermodynamics.

There, it has been translated into structural conformation and polymorphism, where certain systems are more homogeneous, containing simple and ordered structures e.g. vesicles, while others are more heterogeneous, containing various and deviant between them structures e.g. vesicles, worms and their intermediate phases. The latter could be considered the disordered product of a more entropic process, where nevertheless, equilibrium has been achieved. Of course, there is also nanothermodynamics, which is non-statistical and is present on the nanoscale structures' interface with the aqueous medium, producing membrane effects, such as molecule lateral diffusion and phase separation even after macroscopic equilibrium has occurred[13].

Conclusions

As far as we are concerned, this is the first time that an approach to drug delivery nanosystems based on these theories is proposed. Nowadays, more than ever, it is believed that the understanding of self-assembly, by integration of various scientific concepts, will assist us in understanding nature's function and the creation of life itself[5,14,15].

This can only be accomplished if we comprehend the individual biomaterial properties, along with the lyotropic effect, mainly the concentration of the used biomaterials, which determine the informational/entropic balance and the pathway to be followed during the self-assembly of biomaterials and the degree of arising structural polymorphism.

Biophysics and Thermodynamics are the scientific blocks on which drug delivery nanosystems are built, while the metastable phases play a key role in the biophysical behavior of nanocarriers. Information defines biophysics, where metastable phases play a key role, while entropy is associated with system thermodynamics, as introduced in the second law of thermodynamics. By combining these scientific blocks, we can build innovative drug delivery nanosystems with such complexity that promotes functionality and effectiveness. The information/entropy balance of a nanosystem determines the decision that follows hierarchical criteria for the production of "living morphologies", regarding the kinetic and thermodynamic stability. Those are able to promote processes within phospholipid bilayers i.e. the rate of the release of the drug, which is a process that corresponds to the pharmacokinetic profile of the medicine and consequently, to its effectiveness. □

Ο Ρόλος της Σχέσης Πληροφορίας/Εντροπίας στην Αυτο-οργάνωση. Η Δομική Ιεράρχηση στα Χιμαιρικά Νανοσυστήματα Μεταφοράς Φαρμακομορίων

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Περίληψη

Τα χιμαιρικά/μικτά νανοσυστήματα αποτελούν το επόμενο βήμα στην προσπάθεια αναπτύξεως "έξυπνων" φαρμάκων. Συνδυάζουν διαφόρων τύπων βιοϋλικά, με στόχο να ξεπερασθούν οι περιορισμοί της μεταφοράς φαρμακομορίων. Στην παρούσα μελέτη, προτείνεται μια προσέγγιση επί του σχηματισμού και της λειτουργικότητας των χιμαιρικών νανοσυστημάτων, η οποία βασίζεται στη σχέση πληροφορίας/εντροπίας. Ο συνδυασμός νανοϋλικών οδηγεί σε πολυπλοκότητα των συστημάτων και ως εκ τούτου, μεταβάλει το ενεργειακό μονοπάτι το οποίο ακολουθείται κατά την αυτό-οργάνωση, σύμφωνα με τις ιδιότητες και την περιεχόμενη πληροφορία των μορίων αυτών.
Ενδιάμεσες φάσεις (δηλ. μετασταθείς) φαίνεται να λαμβάνουν ρόλο στη δημιουργία των τελικών μορφολογιών. Τέτοιες προσεγγίσεις δύναται να συνεισφέρουν στην κατανόησή μας περί των μηχανισμών της φύσεως, ενώ ταυτοχρόνως μας οδηγούν ένα βήμα εγγύτερα στην ανάπτυξη αποτελεσματικότερων και ασφαλέστερων συστημάτων μεταφορά φαρμακομορίων.

ΛΕΞΕΙΣ-ΚΛΕΙΔΙΑ: Χιμαιρικά νανοσυστήματα, Βιοϋλικά, Αυτο-οργάνωση, Πληροφορία/εντροπία

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Modern strategies for photothermal and ionizing radiation therapy based on gold nanoparticles-mediated radiosensitization

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Abstract

Gold nanoparticles are still to the forefront of cancer research due to their ability to act both as radiosensitizers and photothermal sensitizing agents. The rapid development of the nanotechnology boosts the growth of a wide variety of gold nanoparticles regarding the shape (nanoshells, nanorods, nanocages etc), the size and the surface functionalization. Here, we review some of the recent advances on the use of gold nanoparticles in radiotherapy and in photothermal therapy and the different approaches which have been followed *in vitro* and *in vivo*.

KEYWORDS: Gold nanoparticles; Photothermal therapy; radiotherapy

1. Introduction

A number of research works are published on different types of gold nanoparticles and their applications in targeted cancer therapy radiotherapy and in photothermal treatment[1-5]. Gold nanoparticles (AuNPs) are promising radiosensitizers since they can increase tumor's susceptibility to radiation. High atomic number (Z) materials as gold have the ability to absorb more energy per unit mass than low-Z materials such as water or soft tissues and therefore can enhance the efficacy in radiotherapy[6]. Besides, their excellent electronic properties, AuNPs provides unique optical properties. AuNPs can strongly interact with light and convert the energy of the electromagnetic radiation into heat via a non-irradiation process causing photothermal ablation of tissues. At a specific light wavelength, the electrons of their gold surface can resonate with the electromagnetic field causing a phenomenon called surface plasmon resonance (SPR) which results in strong extinction of light.

Their good biocompatibility with biological macromolecules (e.g amino acids, proteins, DNA) and their easy-surface functionalization make them very promising probes in drug delivery, radiotherapy (RT) and photothermal therapy (PTT). However, there are still several extra-and intracellular barriers which need to overcome so as to move from bench to bedside. The size, the concentration, the shape, the surface-functionalization of the AuNPs are critical parameters for their suc-

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cessful accumulation in their tumour tissue, the cell surface binding, the cellular uptake and the subcellular targeting to organelles such as nuclei or mitochnodria[7]. These parameters are combined with the irradiation conditions such as the energy of radiation (e.g protons, kVp photons and MV photons), the irradiation dose and time, the laser intensity and pulse duration. Thus, many different strategies have been developed to succeed efficient cancer cells damage, to limit the side effects and to improve the effectiveness of RT and PTT.

2. The use of gold nanoparticles in cancer therapy

2.1 The combination of gold nanoparticels with radiotherapy.

Several *in vivo* and *in vitro* preclinical studies have been conducted regarding the applications of gold nanoparticles in radiation therapy, including a range of different external beam radiations types, including kilovoltage (kV) and megavoltage (MV) photons, MeV electrons and heavy charged particles[8-10]. However, local failure after irradiation remains a challenge due to the intrinsic resistance of tumour cells to radiation associated mainly with proteins upregulations[11].

Integrin receptor $\alpha_v\beta_3$ has associated with tissue radioresistance through the upregulation of integrin expression by radiation. Argine (R)-glycine (G)-aspartate (D) (RGD) is an effective peptide existed in many extracellular matrix proteins which can interact with integrin receptors αvβ3 at focal adhesion points. Gold nanorods functionalized with RGD peptide show a dose enhancement in radiotherapy of melanoma in cancer cells[12]. RGB-GNRs (length 44.4 ± 4.7 nm, width 15.1 ± 1.7 nm) under 6 MV irradiation at 4 Gy enhance remarkably the radiosensitivity of cells with a dose modifying factor of 1.35, and enhanced radiation-induced apoptosis. Gold nanospheres conjugated with tumor necrosis Factor-α (size 27 nm) combined with single or fractionated high-dose radiation therapy have reduced effectively the tumor interstitial fluid pressure (IFT) in 4T1 murine breast tumour model[13].

2.2 The combination of gold nanoparticels with photothermal therapy.

The PTT is a biophotonic modality for cancer treatment with minimal invasiveness which attracted new interest due to its combination with functionalized nanoparticles.

Gold nanospheres coated with tumor necrosis factor-alpha (Au-TNF) and heated by nanosecond laser pulses at 690 nm and with energy fluence 0.5 J/cm2 reveal high therapeutic efficiency against murine carcinoma[14]. The Au-TNF conjugates were initially designed to act as drug delivery vehicles to target the solid tumors via enhanced permeability and retention and are already in phase I humans in trial as nanodrugs. However, the photothermal activation of Au-TNF depending on laser parameters (e.g., wavelength, energy, and pulse width) can lead to single or multi dynamic phenomena such as temperature increase, thermal expansion, explosion and fragmentation, formation of acoustic waves, nanobubbles and microbubbles[14]. This synergetic action can open new avenues to the clinical cancer treatment.

Anti-EGFR-conjugated gold nanorods (antiEGFR-GN) under near infrared irradiation at 820 nm and 1.5 W/cm2 for 3 min, exerted synergetic anti-proliferative and apoptotic actions through upregulation of HS70 protein and cleaved caspase-3, downregulation of Ki-67 and EGFR, and inhibition of several intracellular signaling molecules (mTOR, AKT, ERK1/2 and FAK)[15].

Recently, gold nanoshells camouflaged with macrophage cell membrane (MPCM) relieve improved tumoritropic accumulation by recognizing tumor endothelium. MPCM-AuNss achieved high efficiency to suppress tumor growth in mice and selectively ablate cancerous cells under laser irradiation of the tumour area at 808 nm and at 1 W/cm^2 for 5 min[16].

Conclusions

The gold nanoparticles demonstrate to have a multimode role in PTT and RT as photothermal and radiosensitizers agents and also as nanodrugs. However, there is still plenty research ground for studying their potential applications in order to overcome the limitations for their successful entrance in the clinical praxis. □

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