Journal of Physical Chemistry & Biophysics

Stamm et al. J Physic Chem Biophysic 2012, 2:1
DOI: 10.4172/2161-0398.1000105

Research Article Open Access

Functionalization of Ceramic Liposomal Nanoparticles, Cerasomes, with Antibodies

M.T. Stamm[1], Z. Zha[1,2], L. Jiang[1], Z. Dai[2] and Y. Zohar[1]*

[1]*The University of Arizona, Tucson, Arizona, USA*
[2]*Harbin Institute of Technology, Harbin, China*

Abstract

Ceramic nanoparticles and silica microparticles are functionalized with antibodies on their surfaces that act as targeting ligands. For the ceramic liposomal nanoparticles, cerasomes, this is achieved by using the siloxane network present on the cerasome surface as the foundation for chemical treatment processes previously developed for silicon surfaces. The bio-functionality and physical integrity of the cerasomes are characterized, demonstrating successful immobilization of antibodies on the cerasome surface. The surface functionalization allows the cerasomes to deliver drugs to targeted cells expressing certain types of receptors with desired selectivity and specificity that are not possible using standard liposomes. The Silica microparticles are used to mimic cerasomes in experiments targeting cancer cells and the particle-cell specific binding due to the bio-functionalization process is demonstrated.

Keywords: Cerasome; Liposome; Ceramic liposome; Cell targeting; Specific binding; Functionalized nanoparticle

Introduction

Liposomes are vesicles consisting of a hydrophobic lipid bilayer membrane mimicking that of many kinds of biological cells. Since their first creation in the 1960's, liposomes have been researched and utilized extensively. The primary application of liposomes lies in drug delivery [1,2]. Liposomes can be made to encapsulate drugs by forming the vesicles in a solution containing the drugs. The encapsulated solution is able to pass through the membrane only by way of passive diffusion, so the contents are retained for extended periods of time while the liposome is in a suspension. The most common mechanisms of drug delivery by liposomes are passive diffusion through the membrane and uptake of the vesicles by other lipid bilayer structures [3].

Treatment of diseases such as cancer usually requires the administration of large doses of drugs. The inability of a drug to target only tumor cells results in healthy cells being exposed to harmful effects. The use of nanoparticles with the abilities to carry a payload of drugs within their cores and to possess "targeting" molecules on their surfaces is widely viewed as an ideal solution to many of the adverse effects of drug therapies [4].

Using liposomes for drug delivery has the advantage that the drugs are directed to cells and consequently, drastically reduces the amount of drug introduced to the surrounding medium (e.g. blood). Another advantage is due to the enhanced permeability and retention effect and results in liposomes accumulating more in tumor tissue than in normal tissue. However, the mechanism by which liposomes attach to cells results in very low targeting selectivity and specificity [5], which leaves any healthy cells (or lipid bilayer containing structure) vulnerable [6,7]. In order to deliver drugs and target a specific type of cell, an alternative binding mechanism must be employed.

A novel class of liposomal organic-inorganic hybrid nanoparticles known as 'cerasomes' (for ceramic liposome) has recently been developed [6,8,9]. Cerasomes have a liposomal bilayer structure but also have a siloxane network on their spherical external surface (hence the descriptor 'ceramic'). Consequently, cerasomes possess characteristics and potential applications similar to those of liposomes, e.g. bio-compatibility and drug delivery applications. In addition, the siloxane network present on cerasomes may be exploited for surface functionalization purposes. Moreover, the siloxane network on the surface adds rigidity and thus overcomes the inherent morphological instability of liposomes [10], which is a serious problem in practical applications [8,11]

In this work, we present a method of modifying the cerasome external surface based on a bio-functionalization assay such that the vesicle no longer indiscriminately targets any lipid bilayer, but instead is able to target a specific type of cell. We capitalize on the presence of the siloxane network on the external surface of a cerasome by utilizing technologies and processes previously developed for silicon surfaces to functionalize the cerasome surface. This is achieved by immobilizing antibodies on the cerasome surface that serve as targeting ligands [12]. The antibodies on the surface of a drug-containing cerasome recognize and bind to targeted receptors on a particular type of cell and therefore allow drug delivery with improved selectivity and specificity. Due to the high cost of cerasomes and inherent difficulties in working with them, we employ silica particles to mimic the cerasomes in our initial studies of cell-particle interaction. In doing so, we demonstrate the specific binding capabilities of functionalized particles.

Lipid Synthesis and Cerasome Formation

Several stages are involved in synthesizing the organotrialkoxysilane lipids as described elsewhere [13]. Cerasomes can then be realized in aqueous media through a combination of sol–gel reaction and self-assembly of the synthesized lipids to yield bilayer vesicles with a siloxane network on the surface [9], as schematically illustrated in Figure 1.

Cerasome formation begins by dissolving the lipids in chloroform.

***Corresponding author:** Y. Zohar, The University of Arizona, Tucson, Arizona, USA, Tel: 15-20-62-68-093, E-mail: zohar@email.arizona.edu

Received January 23, 2012; **Accepted** February 22, 2012; **Published** February 25, 2012

Citation: Stamm MT, Zha Z, Jiang L, Dai Z, Zohar Y (2012) Functionalization of Ceramic Liposomal Nanoparticles, Cerasomes, with Antibodies. J Physic Chem Biophysic 2:105. doi:10.4172/2161-0398.1000105

The solvent is slowly evaporated in a flask under continuous rotation such that a thin and uniform layer of lipids is left on the flask wall. The layer of lipids is re-suspended in water using ultrasonic excitation. The lipids in suspension spontaneously self-assemble into nanoparticles with a bilayer vesicle structure and cross-link at the lipid head-groups over a period of several days at approximately 4°C. The cross-linking stabilizes the vesicle against breakdown into its constituent lipids and adds rigidity to the membrane. After the completion of cross-linking, the cerasomes are robust; no obvious changes to their structure have been observed due to processes such as ultrasonic excitation. The siloxane network on their external surface provides the required surface properties for subsequent chemical processes. Specifically, the silicon atoms allow many of the processes developed for silicon substrates to be applied for manipulation of the cerasome properties.

The morphology of the obtained cerasomes was examined using a Scanning Electron Microscope (SEM) to verify their successful formation, as well as for later comparison with functionalized cerasomes. A small volume of the cerasome suspension was dropped on the surface of a silicon die. The suspending DI water in the sample was air-dried at room temperature and following standard dehydration and critical point drying steps, was subsequently coated with an approximately 15 nm-thick layer of platinum using sputtering before the imaging. A typical SEM image of the cerasomes is shown in Figure 2. The cerasomes are seen to be roughly spherical in shape with a diameter ranging from 200 nm to 500 nm. Most of them are isolated individuals although, occasionally, intact cerasome pairs were observed. This may be caused by the air-drying process, during which cerasomes are pulled together by capillary force in the suspending medium. It is also noticed from the SEM image that the cerasomes appear not perfectly rounded with local dips on the surface. Irregularities are expected with self-assembling and/or biological specimens. Figure 3 is a close-up image of a nicely formed cerasome prior to functionalization. The external surface is seen to be very smooth, which demonstrates that the structure is a self-assembled and closed membrane of lipid bilayer as opposed to an agglomeration of lipids or fragments of a lipid bilayer.

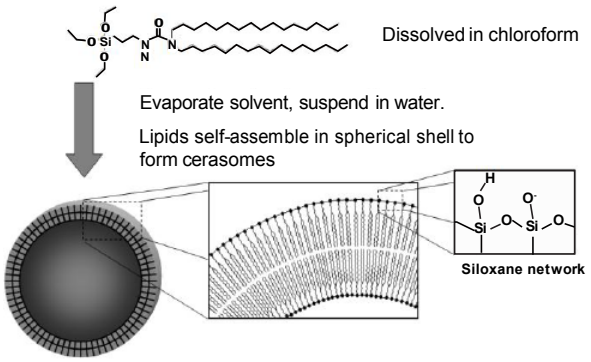

Figure 1: A schematic illustration of cerasome formation from organotrialkoxysilane lipids.

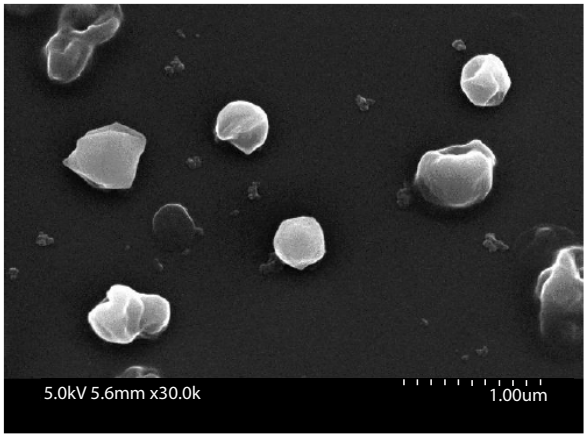

Figure 2: A SEM image of a group of cerasomes after formation and prior to functionalization.

As-fabricated cerasomes vary in size over a wide range. The size distribution of cerasomes in a suspension was obtained utilizing the Dynamic Light Scattering (DLS) technique using a Zetasizer nano ZS90 (Marvern Instruments). Each cerasome sample was scanned using a cuvette containing 350 µl of cerasome suspension at 20°C. Average scattering intensity data at a scattering angle of 90° were collected with a measurement time of 10 sec for each scan and about 20 scans for each sample. Figure 4 shows the size distribution of an original cerasome sample obtained from the DLS analysis. The size distribution follows a log-normal statistical model; it consists of particles varying over wide range in hydraulic diameter, between approximately 80 and 500 nm with its most probable diameter around 160nm. Cerasomes with a certain size can be obtained using size-exclusion filtration. As an example, the size distribution of a filtered sample is shown in Figure 4, where 200nm range filtration had been performed on a sample using gel-filtration chromatography. The size distribution of the filtered sample seems to follow a normal distribution; the average cerasome diameter in the filtered sample is about 205nm with a standard deviation of 8nm.

Clearly, the bulk of the fabricated cerasomes is less than 500nm in diameter, as seen in Figure 4; however, it is difficult to observe these cerasomes using standard optical microscopy. Since the cerasome surface properties of interest are independent of size, larger particles 500-1000nm in diameter were selected in the present work for demonstration of the functionalization process. These cerasomes were fluorescently labeled and could be detected using a fluorescence microscope so as to provide clear indications of modified surface properties.

Cerasome Functionalization with Antibodies

To immobilize targeting ligands on a cerasome surface, we followed a protocol that has been successfully utilized in immobilizing antibodies on silica nanoparticles since both cerasomes and silica particles feature surface silanol groups. A similar immunoassay has been detailed in functionalizing microchannels with antibodies [14,15] and the functionalization process used in this work is illustrated in Figure 5. Cerasomes were immersed in a 1% (vol/vol) 3-aminopropyltriethoxysilane (APTES)-acetone solution for 30 min at room temperature. The aminated surface was then activated with a solution of 2% (vol/vol) glutaraldehyde in deionized (DI) water for two hours to promote a Schiff-base reaction between the amine and aldehyde groups. After thoroughly washing with DI water, the cerasomes were incubated in a recombinant protein G solution at a concentration of 50 µg/ml in 1× phosphate buffed saline (PBS) at approximately 4°C for three hours. In order to block excess silanol sites, the cerasomes were washed and then immersed in bovine serum albumin (BSA) solution

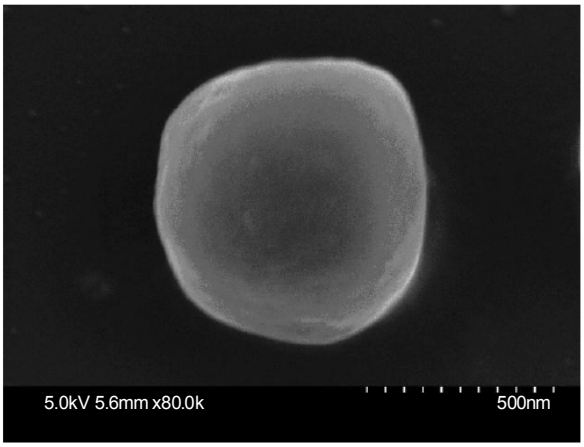

Figure 3: A close-up SEM image of a single cerasome prior to functionalization.

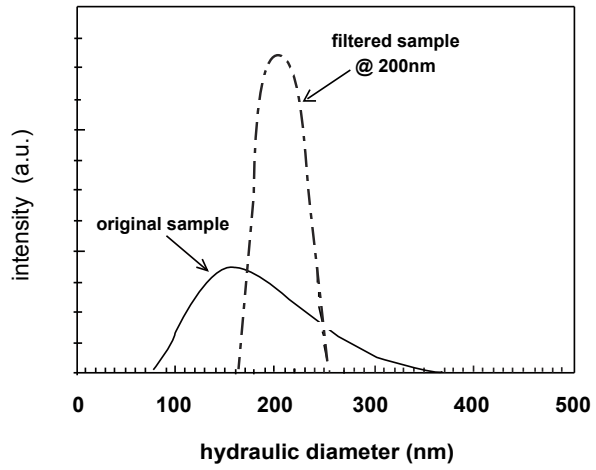

Figure 4: Examples of cerasome size distributions of an original sample and a filtered sample measured by dynamic light scattering method.

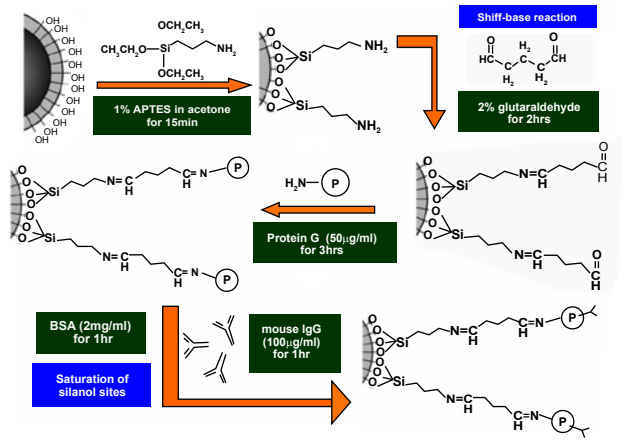

Figure 5: A schematic depiction of the immobilization process for functionalizing cerasomes with antibodies.

of 2 mg/ml concentration for one hour at room temperature. Following a thorough wash with 1×PBS, the assay was completed by incubating the cerasomes with fluorescently tagged Cy3-anti-mouse IgG (from donkey) antibodies at a concentration of 100 μg/ml for one hour at room temperature in a darkened environment. The cerasomes were washed once more with 1× PBS and stored in 1× PBS at approximately 4°C in the dark.

As an alternative to this protocol, the antibody may be attached immediately following the surface activation with glutaraldehyde. The silanol and aldehyde sites are then saturated with BSA and the protein G step is omitted entirely. The glutaraldehyde solution may also be made slightly acidic to increase electrostatic repulsion between aminated particles [16] during the reaction and inhibit bridging/aggregation [17,18]. The result of this alternate protocol is that the antibodies are not oriented with the specific binding region outward [19]. As long as the target cells are Fc-blocked, however, this will not lead to non-specific binding.

Characterization of Bio-Functionalized Cerasomes

The fluorescently tagged antibodies were used in the assay as a means to verify the successful functionalization of the cerasomes. If the antibodies were indeed immobilized on the cerasome surfaces, then there should be an observable difference between unmodified and the functionalized cerasomes when observed using a fluorescence microscope (Nikon Eclipse 80i) with proper excitation and emission. Fluorescence microscope images of cerasomes before and after functionalization are compared in Figure 6. Images of two cerasome samples are shown under white light in Figure 6 (A1) and (B1), while those in Figure 6 (A2) and (B2) are taken at the same locations but using 550 nm excitation and 570 nm emission filters in order to detect the Cy3 labels. The images in Figure 6 (A1) and (A2), taken for unmodified cerasomes, clearly show the presence of cerasomes under white light but show no detectable fluorescence signal using the proper excitation/emission filters. The images in Figure 6 (B1) and (B2), taken for functionalized cerasomes, show a number of cerasomes under white light and fluorescence signals at the same identical locations using the excitation/emission filters. The results indicate that the fluorescently tagged antibodies have been successfully immobilized on the cerasome surfaces.

With the antibodies immobilized on the surface, the cerasomes are functionalized and have the ability of targeting specific cell types based on the receptors they express. Although anti-mouse IgG was used as an example antibody in this work, the functionalization method could be implemented to immobilize virtually any antibody on the cerasome surface. This gives the cerasomes a potential of targeted drug-delivery to specific cell types, enhancing the selectivity of the therapeutic treatment and minimizing side effects.

To examine whether the cerasomes were damaged by the successive chemical processing steps, SEM images (not shown here for brevity) of cerasomes were taken after the conclusion of the entire procedure of surface functionalization. The images, similar to the one shown in Figure 3, confirm that functionalized cerasomes are still intact as indicated by the retention of their spherical shape and smooth surface. The retention of the nearly spherical shape shows that the various functionalization steps have not significantly affected the morphological properties of the cerasomes in any adverse way. The smooth surface of the functionalized cerasomes, as that of the unmodified ones, indicates that the vesicle surfaces are fully-enclosed shells rather than an agglomeration. The cerasome membrane closure

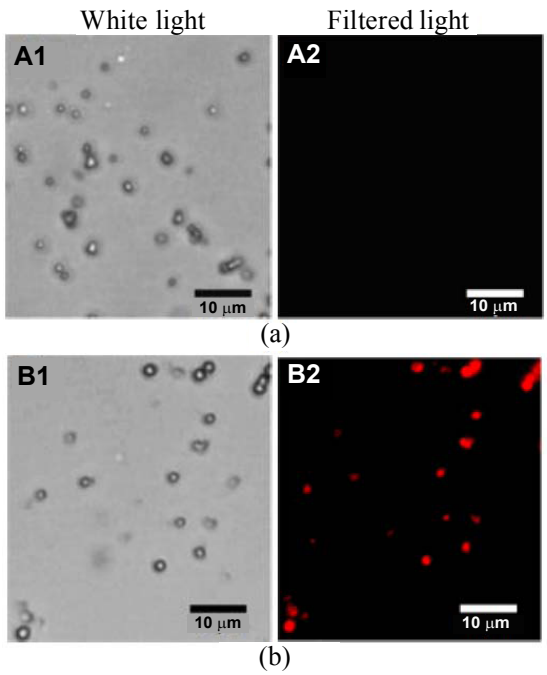

Figure 6: Micrographs of cerasomes under white light (A1 and B1) and filtered light (A2 and B2) taken: (a) before, and (b) after functionalization with antibodies.

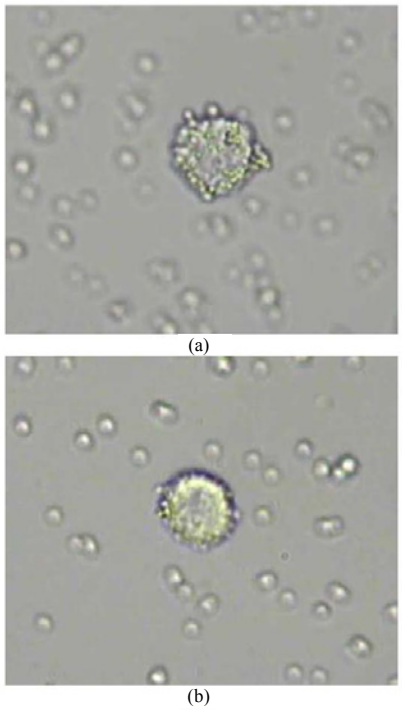

Figure 7: A typical EpCAM-expressing target cancer cell (BT-20) after exposure to particles functionalized with: (a) the proper counter-receptor anti-human EpCAM, and (b) anti-mouse IgG, demonstrating the specific binding capabilities of the functionalized particles. All other experimental conditions were identical.

is a critical requirement for vesicles to be used as drug delivery vehicles since an opening would cause dispersal of any encapsulated contents into the surrounding medium.

Functionalized Particle - Target Cell Interaction

In order to confirm the ability of functionalized particles to specifically bind to target cells, experiments were conducted with functionalized silica microparticles and BT-20 cancer cells. The BT-20 line of cells expresses the epithelial-cell-adhesion molecule (EpCAM), which is over-expressed in many tumor cells but is absent in normal blood cells [20]. Hence, EpCAM was chosen as the target receptor in these experiments. Silica microparticles approximately 1.5 μm in diameter were functionalized with two types of antibodies: anti-mouse IgG and anti-human EpCAM. The BT-20 cancer cells were then Fc-blocked and added to suspensions of functionalized microparticles in 1x PBS containing 1% FBS. Each suspension was placed in a well on a 24-well plate mounted on a shaking incubator for 20 min at 25°C. Next, the mixed suspensions were dispensed on microscope slides allowing the particles and cells to settle onto the surface of the slides before imaging. The particle concentration was sufficiently low to prevent significant numbers of particles from settling onto the cell surfaces. Figure 7 shows typical cells from two types of particle-cell suspensions subjected to identical experimental conditions. A typical cell from the suspension consisting of particles functionalized with anti-mouse IgG and BT-20 cancer cells is shown in Figure 7a, while a similar cell from a suspension consisting of particles functionalized with anti-human EpCAM is shown in Figure 7b. Clearly, the particles functionalized with the anti-human EpCAM result in substantial particle-cell specific binding, while the particles functionalized with anti-mouse IgG show very little particle-cell nonspecific binding. For the control group (particles functionalized with anti-mouse IgG), on the average, fewer than 10 particles were observed to be bound to a target cell; in contrast, more than a hundred particles per target cell were observed to be specifically bound for the test group of particles functionalized with the targeting ligand (anti-human EpCAM).

Conclusions

A novel class of ceramic liposomal nanoparticles, cerasomes, has been functionalized with fluorescently-tagged antibodies on their surfaces. The bio-functionality of the cerasomes has been confirmed by examining the presence of the antibodies on the cerasome surface using fluorescence microscopy. No obvious changes are observed, based on the SEM microscopy, in size and shape of the functionalized cerasomes compared with unmodified ones, demonstrating the integrity of the functionalized cerasomes. This functionalization method, in general, is not limited to the use of a particular type of antibodies. Therefore, the method described herein can be used to create functionalized cerasomes tailor-made for any target cell type by immobilizing proper counter-receptor antibodies. The ability of functionalized particles to specifically bind to target cells has been demonstrated. Particles functionalized with the proper counter-receptor showed a significantly increased attachment efficiency to target cancer cells over particles functionalized with other antibodies. The cerasomes with immobilized antibodies, which act as homing ligands, promise great potential of realizing multi-purpose smart vehicles for pin-point delivery of an array of payloads at a nanoscale with significantly improved selectivity over standard liposomes.

Acknowledgments

This study is supported by an NSF grant # 0943321.

References

1. Cavalcanti L, Konovalov O, Torriani I, Haas H (2005) Drug loading to lipid-based cationic nanoparticles. Nucl Instrum Methods Phys Res Sect A 238: 290-293.
2. Lian T, Ho RJY (2001) Trends and developments in liposome drug delivery systems. J Pharm Sci 90: 667-680.
3. Pathak P, Katiyar VK (2007) Multi-functional nanoparticles and their role in cancer drug delivery-A Review. 3: 3-17.
4. Conrad D (2006) Tumor-seeking nanoparticles. NCI Alliance for nanotechnology in cancer, September monthly feature 1-3.
5. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D (1999) Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharmacol Rev 51: 691-744.
6. Cao Z, Ma Y, Yue X, Li S, Dai Z, et al. (2010) Stabilized liposomal nanohybrid cerasomes for drug delivery applications. Chem Commun 46: 5265-5267.
7. Jain K (2005) Editorial: Targeted drug delivery for cancer. Technol Cancer Res Treat 4: 311-313.
8. Hashizume M, Kawanami S, Iwamoto S, Isomoto T, Kikuchi J (2003) Stable vesicular nanoparticle 'Cerasome' as an organic–inorganic hybrid formed with organoalkoxysilane lipids having a hydrogen-bonding unit. Thin Solid Films 438-439: 20-26.
9. Katagiri K, Hashizume M, Ariga K, Terashima T, Kikuchi J (2007) Preparation and characterization of a novel organic–inorganic nanohybrid 'cerasome' formed with a liposomal membrane and silicate surface. Chemistry 13: 5272-5281.
10. Yue X, Jing Y, Dai Z (2011) Liposomal cerasome: a nanohybrid of liposome and silica. Asia-Pac. J Chem Eng 6: 569-574.
11. Katagiri K, Hamasaki R, Ariga K, Kikuchi J (2003) Preparation and surface modification of novel vesicular nano-particle 'cerasome' with liposomal bilayer and silicate surface. J Sol-gel Sci Techn 26: 393-396.
12. Van Vlerken LE, Amiji MM (2006) Multi-functional polymeric nanoparticles for tumour-targeted drug delivery. Expert Opin Drug Deliv 3: 205-216.
13. Sasaki Y, Matsui K, Aoyama Y, Kikuchi J (2006) Cerasome as an infusible and cell-friendly gene carrier: synthesis of cerasome-forming lipids and transfection using cerasome. Nat protoc 1: 1227-1234.
14. Lee LM, Heimark RL, Baygents JC, Zohar Y (2006) Self-aligned immobilization of proteins utilizing PEG patterns. Nanotechnology 17: S29-S33.
15. Cheung LSL, Zheng X, Stopa A, Baygents JC, Guzman R, et al. (2009) Detachment of captured cancer cells under flow acceleration in a bio-functionalized microchannel. Lab Chip 9: 1721-1731.
16. Butterworth MD, Corradi R, Johal J, Lascelles SF, Armes SP, et al. (1995) Zeta-potential measurements on conducting polymer-inorganic oxide nanocomposite particles. J Colloid Interf Sci 174: 510-517.
17. Gudipaty T, Stamm MT, Cheung LSL, Jiang L, Zohar Y (2010) Cluster formation and growth in microchannel flow of dilute suspension. Microfluid. Nanofluid. 10: 661-669.
18. Stamm MT, Gudipaty T, Rush C, Jiang L, Zohar Y (2011) Particle aggregation rate in a microchannel due to a dilute suspension flow. Microfluid Nanofluid. 11: 395-403.
19. Bae YM, Oh BK, Lee W, Lee WH, Choi JW (2005) Study on orientation of immunoglobulin G on protein G layer. Biosens Bioelectron 21: 103-110.
20. Went PT, Lugli A, Meier S, Bundi M, Mirlacher M, et al. (2004) Frequent EpCAM protein expression in human carcinomas. Hum Pathol 35: 122-128.
21. Stamm MT, Zha Z, Jiang L, Dai Z, Zohar Y (2011) Functionalization of ceramic liposomal nanoparticles, cerasomes, with antibodies. 16th International Conference on Solid-State Sensors, Actuators and Microsystems 226-229.