Journal of Biomaterials and Nanobiotechnology, 2015, 6, 257-266
Published Online October 2015 in SciRes. http://www.scirp.org/journal/jbnb
http://dx.doi.org/10.4236/jbnb.2015.64024

Cytotoxicity, *in Vivo* Skin Irritation and Acute Systemic Toxicity of the Mesoporous Magnesium Carbonate Upsalite®

Sara Frykstrand, Johan Forsgren, Peng Zhang, Maria Strømme, Natalia Ferraz*

Division for Nanotechnology and Functional Materials, Department of Engineering Sciences, The Ångström Laboratory, Uppsala University, Uppsala, Sweden
Email: *natalia.ferraz@angstrom.uu.se

Received 24 August 2015; accepted 11 October 2015; published 14 October 2015

Abstract

Upsalite® is a mesoporous magnesium carbonate synthesized without using surfactants and therefore highly attractive from environmental and production economy points of view. The material has recently been suggested as drug delivery vehicle and as topical bacteriostatic agent. In order to continue exploring these and other bio-related applications of the material, primary biocompatibility studies are needed. Herein we present the first *in vivo* acute systemic toxicity and skin irritation analyses as well as *in vitro* cytotoxicity evaluations of Upsalite®. The material was found to be non-toxic for human dermal fibroblasts cells up to a concentration of 1000 µg/ml and 48 h exposure in contrast to the mesoporous silica material SBA-15, used as reference, which significantly affected cell viability at particle concentration of 500 and 1000 µg/ml after the same exposure time. Topical application of Upsalite® resulted in negligible cutaneous reactions in a rabbit skin irritation model and no evidence of significant systemic toxicity was found when saline extracts of Upsalite® were injected in mice. Injection of sesame oil extract, however, resulted in transient weight loss, most likely due to injection of particles, and not toxic leachables. The presented results form the basis for future development of Upsalite® and similar mesoporous materials in biomedical applications and further toxicity as well as biocompatibility studies should be directed towards specific areas of use.

Keywords

Mesoporous, Magnesium Carbonate, Cytotoxicity, Skin Irritation, Acute Systemic Toxicity

*Corresponding author.

How to cite this paper: Frykstrand, S., Forsgren, J., Zhang, P., Strømme, M. and Ferraz, N. (2015) Cytotoxicity, *in Vivo* Skin Irritation and Acute Systemic Toxicity of the Mesoporous Magnesium Carbonate Upsalite®. *Journal of Biomaterials and Nanobiotechnology*, **6**, 257-266. http://dx.doi.org/10.4236/jbnb.2015.64024

1. Introduction

Mesoporous inorganic solids with narrow pore size distributions have been subjected to extensive research in recent years [1]. Biomedicine is an emerging application field for these materials, for which a large number of potential areas of use has been investigated [2], e.g. for bone regeneration [3], as vaccine adjuvants [4] [5] and in drug delivery [6]-[8]. Such application fields place high demands on the materials to be used. Not only are the materials required to show exceptional properties that can offer new and better solutions compared to presently used materials, but they also have to be biocompatible. The desired material properties of mesoporous materials to be utilized in many of these applications are the large internal volumes and the high surface areas allowing for high-capacity adsorption of different molecules, such as drugs and proteins [9] [10]. The requirement on biocompability includes two principal elements: the absence of cytotoxic effects and the desired functionality [11]. Cytotoxicity mainly concerns the survival of cells and the maintenance of specific cellular functions under the influence of the material. Functionality relies on the absence of impairment of cellular function and requires that the mechanical, chemical and physical features of the material are sufficient for the performance of cell-specific functions [11]. In recent years, due to rapid development of new biomaterials, the scope of biocompatibility has been widely broadened to include *in vivo* toxicity and changes of cells at molecular and histological levels [12].

In general, mesoporous materials are derived from supramolecular assemblies of surfactants which template the inorganic component (commonly silica) during synthesis [13]-[16]. In order to form the final mesoporous material, the surfactants have to be removed. This is commonly done by pyrolysis or dissolution with appropriate solvents [13]. Surfactants are often both expensive and toxic organic molecules that require high temperatures and/or additional chemicals upon removal from the materials. As well, their presence during synthesis rules out the possibility of *in-situ* loading of functional agents in the mesopores [16] [17]. This adds cost and environmental burden from the production of such materials; therefore there is a need for novel synthesis approaches for mesoporous materials that do not require the use of surfactants. Even though there are some examples in the literature of such syntheses [18] [19], the majority of mesoporous materials are still produced using surfactants. In light of this issue, we have recently developed a mesoporous version of magnesium carbonate, Upsalite®, synthesized without the aid of surfactants [20]. Upsalite® has a high surface area and narrow pore size distribution centered around ~5.5 nm [21] and it has recently been shown that the material can work as a solubility enhancer for the model drug Ibuprofen by suppressing its crystallization [6].

The same properties that make mesoporous materials so appealing for biomedical applications can impact the way which they interact with biological systems and new studies are required to assess the biocompatibility profile of the materials. For example, even though mesoporous silica shares similar chemical composition and disordered atomic structure with synthetic amorphous silica, an approved food additive, the high surface area coupled to the ability to adsorb large amounts of biologically relevant molecules within the mesopores demands *de novo* evaluation of the safety profile of the material [17]. The same argumentation should be used for Upsalite®, *i.e.* despite magnesium carbonate being listed as GRAS (generally recognized as safe) by the U.S. Food and Drug Administration (FDA) and having an E-number (E504) [22], the porous structure, high surface area and adsorption properties of Upsalite® [20] call for the need for toxicity evaluation as a key element in the development of novel bioapplications of the material.

Herein we present a first toxicity screening of Upsalite®, where the material is analyzed for *in vitro* cytotoxicity using a human dermal fibroblast cell line, for *in vivo* skin irritation in rabbits and for *in vivo* acute systemic toxicity in mice.

2. Materials and Methods

2.1. Chemicals and Reagents

Magnesium oxide (MgO, >99%), methanol (>99.8%), sesame oil and culture medium (DMEM-F12) were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS) was purchased from VWR, carbon dioxide (CO_2, N48) was purchased from Air Liquid and mesoporous silica (SBA-15, >99.0%) was purchased from ACS Material, LLC. All reagents were used without further purification.

2.2. Synthesis of the Mesoporous Magnesium Carbonate Upsalite®

Two syntheses of Upsalite® were carried out as described previously [20] [21] but on a larger scale. 2.5 l me-

thanol was mixed with 170 g MgO at 500 rpm in a 5 l Ecoclave pressure reactor (Büchi). The reaction was carried out at 55˚C under 3 bar CO_2 pressure. After four days reaction time, the temperature was decreased to room temperature and the reactor was depressurized. The product was dried at 75˚C using a rotary evaporator and then calcined at 250˚C for 12 h.

After calcination, the material was grinded in a mortar to reduce the particle size. Thereafter two different sieves, 200 µm and 50 µm, were used to sieve the grinded material in order to obtain samples with a controlled particle size distribution, between 200 and 50 µm. The materials were characterized after calcination and grinding. Two different batches of material with similar characteristics were used in this study.

For the *in vitro* and *in vivo* studies the synthesized Upsalite® particles and the reference material mesoporous silica SBA-15 (particle size 1 - 2 µm) were sterilized by dry heat (3 hours at 180˚C) [23].

2.3. Material Characterization

Nitrogen sorption measurements were carried out at 77 K using an ASAP 2020 from Micromeritics. The samples were degassed at 95˚C under vacuum for 10 h prior to analysis with a vacuum set point of 10 µm Hg. The specific surface area (SSA) was determined by applying the Brunauer-Emmet-Teller (BET) equation [24] to the relative pressure range 0.05 - 0.30 of the adsorption branch of the isotherm. The BET calculations were performed with the ASAP 2020 V3.04 software from Micromeritics. The pore size distribution was determined using the DFT method carried out with the DFT Plus software from Micrometrics using the model for nitrogen adsorption at 77 K for slit-shape geometry with non-negative regularization. The standard deviations of the DFT fits were between 1.18900 and 3.77182 cm^3/g, STP for the recorded isotherms.

Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (*ATR-FTIR*) was performed with a Bruker Tensor 27 instrument using a Platinum ATR diamond cell. A background scan was recorded prior to the measurement and subtracted from the sample spectrum, 32 scans were signal-averaged for each spectrum.

Helium pycnometry was performed on the samples in order to determine the true density. Six measurements were performed on each sample ($n = 6$) using an instrument from Micromertics, USA, model AccuPyc 1340.

Scanning Electron Microscopy (*SEM*) was performed using a Leo 1550 instrument equipped with an in-lens detector to study the morphology of the materials. Prior to the analysis the samples were sputter coated with a thin layer of gold/palladium.

2.4. *In Vitro* Cytotoxicity

The cellular response induced by Upsalite® was investigated in a direct cell test using human dermal fibroblasts (hDF). The cells were cultured in DMEM-F12 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 IU/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine at 37˚C, 5% CO_2 in a humidified atmosphere. Cells in passage 5 - 15 were harvested using trypsin-EDTA treatment and counted using a hemocytometer. Cell viability was assessed using trypan blue staining (90% - 99% viable cells). Cell suspension in culture medium was prepared at a density of 40,000 cells/ml and 20,000 cells/well were added to a 24-well tissue culture plate in triplicate samples. Cells were cultured for 24 ± 2 hours at 37˚C and 5% CO_2, reaching near confluency. After that, the culture medium was removed and fresh culture medium containing 1000 µg/ml, 500 µg/ml, 250 µg/ml and 50 µg/ml Upsalite® material (500 µl per well) was added and cells were incubated for additional 24 or 48 ± 2 hours at 37˚C and 5% CO_2. The negative control was culture medium and the positive control was 5% (v/v) dimethylsulfoxide (DMSO) in culture medium. SBA-15 mesoporous silica served as reference material.

Light microscopy. The material supplemented culture medium was removed from the wells after 24 or 48 ± 2 hours, and the cells were subsequently carefully rinsed two times with PBS. The adherent fibroblasts were observed under a light microscope (Nikon Eclipse TE2000-U) to evaluate the cell number and morphology.

Alamar blue assay. The material supplemented culture medium was removed from the wells after 24 or 48 ± 2 hours, and the cells were subsequently carefully rinsed two times with PBS. 0.5 ml of Alamar blue stock solution (Biosource) diluted 1:10 in PBS was added to the wells and incubated at 37˚C and 5% CO_2 for 90 min. Alamar blue is a non-toxic metabolic indicator for cell growth. Upon uptake into the cell, the dye is reduced and changes from a non-fluorescent to a fluorescent form. The change in fluorescence correlates to the cell proliferation in the sample. Aliquots of 100 µl from each well were transferred to a 96-well plate, and the fluorescence intensity was measured at 560 nm excitation and 590 nm emission wavelength using a spectrofluorometer (Tecan infinte® M200). The results are presented as percentage of cell viability of the negative control. The pre-

sented data are given as mean ±95% confidence interval for $n = 5$.

A control measurement was performed to discard any interaction between the material supplemented medium and the Alamar blue reagent. In this case, the assay was done with Upsalite® and SBA-15 supplemented medium in the absence of cells and the results were compared with the blank. No interactions were detected since no statistically significant difference was found between the fluorescence values from the material supplemented medium without cell contact and those from the blanks.

2.5. *In Vivo* Skin Irritation

Two 2.5 cm × 2.5 cm squares of sterile gauze containing 0.5 g sterile Upsalite® powder and two 2.5 cm × 2.5 cm squares of sterile gauze without material (negative control) were topically applied to the skin of three male rabbits (New Zeeland White) and left in place for 24 hours. 4 to 24 hours prior to the treatment the fur on each rabbit's back were clipped with an electrical clipper. The sites were observed for erythema (redness of skin or rash) and edema (swelling) at 1, 24, 48 and 72 hours after removal of the gauze. The study was conducted according to the ISO 10993 standard, part 10 [25] at NAMSA® contract research laboratory (France) and NIH guidelines for care and use of laboratory animals were followed. The protocol has been approved by NAMSA Ethical Committee.

2.6. *In Vivo* Acute Systemic Toxicity

Sterile Upsalite® powder was extracted for 72 ± 2 hours at 50˚C in stirred solutions of 0.9% NaCl and sesame oil. The ratio between the material and the extraction vehicle was 0.2 g/ml. The extracts were stored at room temperature before use for a maximum of 24 hours, and shaken immediately before use to ensure homogeneity of the extract. Five female mice (OF1 Ico) per extract and per negative control group were injected at a dose of 50 ml/kg using the intrapertioneal (IP) route. The mice were observed for adverse reactions immediately after dosing (T0) and after 4, 24, 48 and 72 hours and were also weighted 24, 48 and 72 hours after the injection. The study was conducted according to the requirements of the ISO 10993 standard, part 11 [26] at NAMSA® contract research laboratory (France) and NIH guidelines for care and use of laboratory animals were followed. The protocol has been approved by NAMSA Ethical Committee.

The presence of leachables in the sesame oil extract was analyzed using ATR-FTIR. A spectrum was recorded for the sesame oil after extraction with Upsalite® particles and compared with a spectrum for the as-received sesame oil.

2.7. Statistical Analysis

Data were evaluated using one-way ANOVA with Origin 9.0 software. Samples were considered statistically different at $p < 0.05$.

3. Results

3.1. Material Characterization

The syntheses of Upsalite® resulted in a white coarse powder that was grinded and sieved, obtaining particles between 50 - 200 µm that were used for the studies. ATR-FTIR confirmed that the material was composed of magnesium carbonate, as also shown in previous work [20] [21]. The final particles were characterized together with the as-received SBA-15 particles, the results from the characterizations are summarized in **Table 1** and SEM images of the particles can be seen in **Figure 1**. As can be seen the materials differ in morphology, while Upsalite® form irregular shaped particles between 50 - 200 µm in size, the SBA-15 particles are all rod-shaped with a particle size of 1 - 2 µm. The densities are ~2.3 cm^3/g for both materials and the pore size is ~5 nm for the Upsalite® particles and ~6 nm for the SBA-15 particles. The surface area and pore volume are slightly larger for the SBA-15 particles than for the Upsalite® particles, see **Table 1**. The two batches of Upsalite® particles have surface areas of 207 and 284 m^2/g and pore volumes of 0.34 and 0.48 cm^3/g, respectively, while the SBA-15 particles have a surface area of 428 m^2/g and a pore volume of 0.62 cm^3/g. Two batches of Upsalite® were used in the study; batch 1 was used for the *in vitro* cytotoxicity evaluation, the *in vivo* skin irritation test and acute systemic toxicity assessment, while batch 2 was used for the ATR-FTIR study of the sesame oil extract.

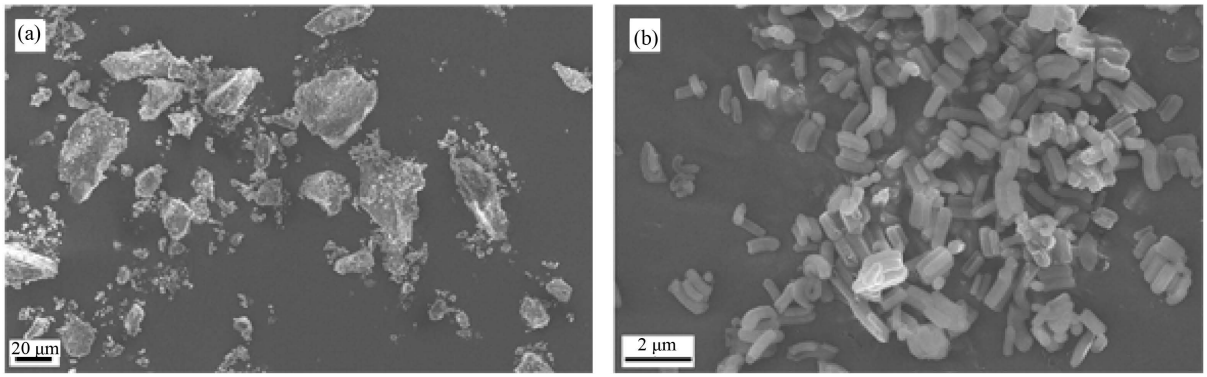

Figure 1. SEM images of grinded and sieved Upsalite® (a) and SBA-15, (b) particles.

Table 1. Material characteristics for Upsalite® and SBA-15 particles used in the study.

Material	Pore volume (cm^3/g)[a]	Surface area (m^2/g)[b]	Pore size (nm)[c]	Density (g/cm^3)[d]	Particle shape[e]	Particle size (µm)[f]
Upsalite® batch 1	0.34	207	5.3	2.28	Irregular	50 - 200
Upsalite® batch 2	0.48	284	5.4	2.31	Irregular	50 - 200
SBA-15	0.62	428	6.3	2.35	Rods	1 - 2

[a]Single point adsorption at $P/P_0 \approx 1$; [b]Established with the BET equation [24]; [c]Established with the DFT method applied to the nitrogen adsorption isotherm; [d]True density, established with He pyconometry; [e]Predominant particle shape in the sample according to SEM; [f]Measured from the SEM images.

3.2. *In Vitro* Cytotoxicity

Direct cell tests were used to determine the viability of fibroblasts in contact with Upsalite® and SBA-15 particles at four different concentrations (1000 µg/ml, 500 µg/ml, 200 µg/ml, 50 µg/ml) and two different time points (24 and 48 hours). Dermal fibroblasts are cells within the dermis layer of the skin which are responsible for generating connective tissue and allowing the skin to recover from injury [27]. Herein we examined whether Upsalite® (50 - 200 µm size) and SBA-15 particles have cytotoxic effects on these cells. SBA-15 is a mesoporous silica developed in 2003 at University of California, Santa Barbara and commercially available today. The average pore size of SBA-15 is 6.3 nm which is similar to the pore size of Upsalite®. The material is well-known and has been used for many different biomedical related applications, *e.g.* as a drug delivery vehicle [13] [28] [29]. *In vivo* studies have shown little toxicity with small quantities of 1 - 2 µm sized SBA particles [30]. For these reasons SBA-15 was considered a well-suited material to be used as a reference when studying the cytotoxicity of Upsalite®.

Form **Figure 2(a)** and **Figure 2(b)**, it can be observed that the cell viability was above the 70% toxicity limit [31] after 24 and 48 hours exposure to 50 - 1000 µg/ml Upsalite® showing that Upsalite® particles do not exhibit cytotoxic effects up to concentrations of 1000 µg/ml. Further, the light microscopy images in **Figure 3** confirm the non-toxic effects of Upsalite®. After 48 hours of exposure to Upsalite®, hDF cells adhered in large number and showed typical fibroblast morphology, comparable with the cell adhesion pattern and morphology observed in the negative control.

Cell exposed to 50 - 1000 µg/ml SBA-15 particle suspensions for 24 h showed no sign of toxicity (**Figure 2(c)**). However, after 48 hours the cell viability was below the 70% toxicity limit for the higher concentrations of SBA-15 particle suspension, 500 and 1000 µg/ml (**Figure 2(d)**). Light microscopy images displayed in **Figure 4** shows that the cell number and morphology were affected when hDF cells were exposed to 500 and 1000 µg/ml SBA-15 particle suspensions for 48 h, thus revealing the cytotoxic effects of SBA-15 at high concentrations and prolonged exposure time.

3.3. *In Vivo* Skin Irritation

No irritation was observed on the skin of the rabbits during the study. The scoring from the observations of skin

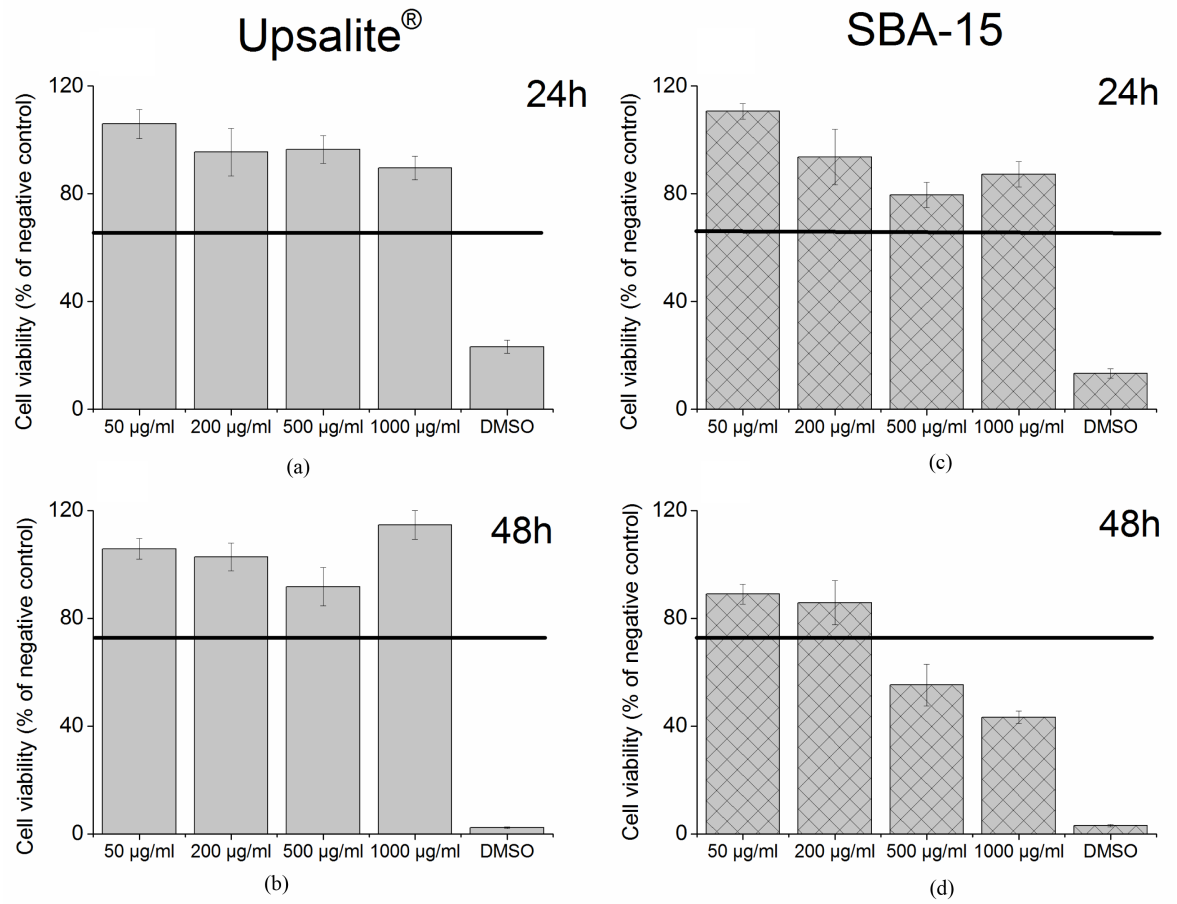

Figure 2. Cell viability of hDF cells exposed to Upsalite® particle suspensions for 24 (a) and 48 (b) hours as well as to SBA-15 particle suspensions for 24 (c) and 48 (d) hours. Data represent mean ±95% confidence interval for $n = 5$. Cell viability values larger than 70% of the negative control indicate a non-cytotoxic effect.

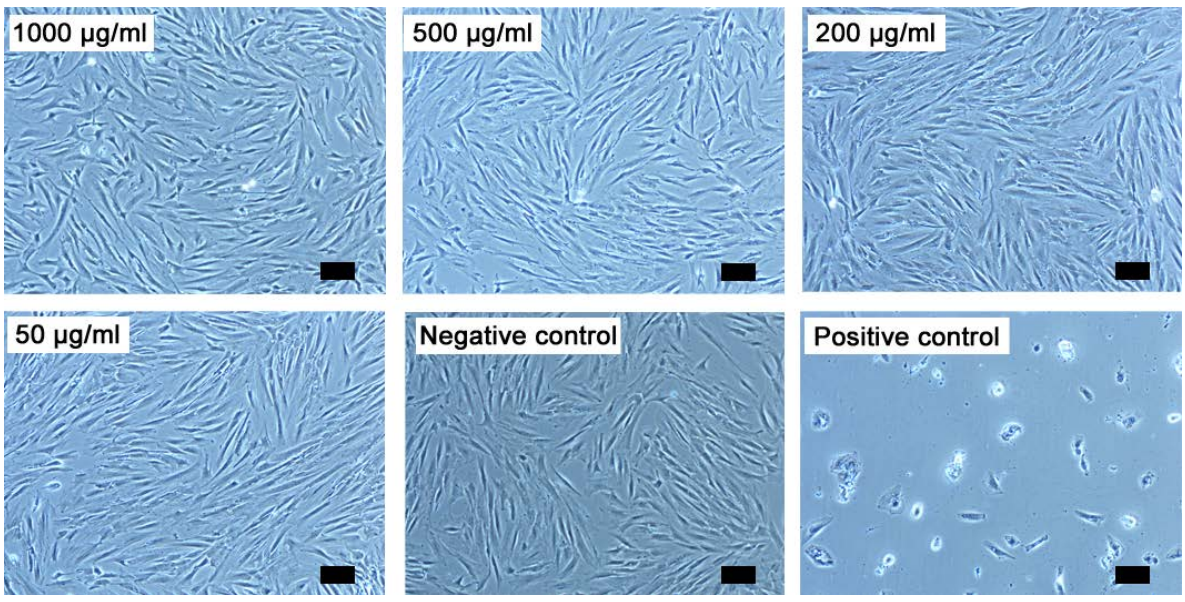

Figure 3. Representative light microscopy images of hDF cells exposed to Upsalite® particle suspensions for 48 hours together with the positive control (5% DMSO) and the negative control (cell culture medium). Scale bars 100 µm.

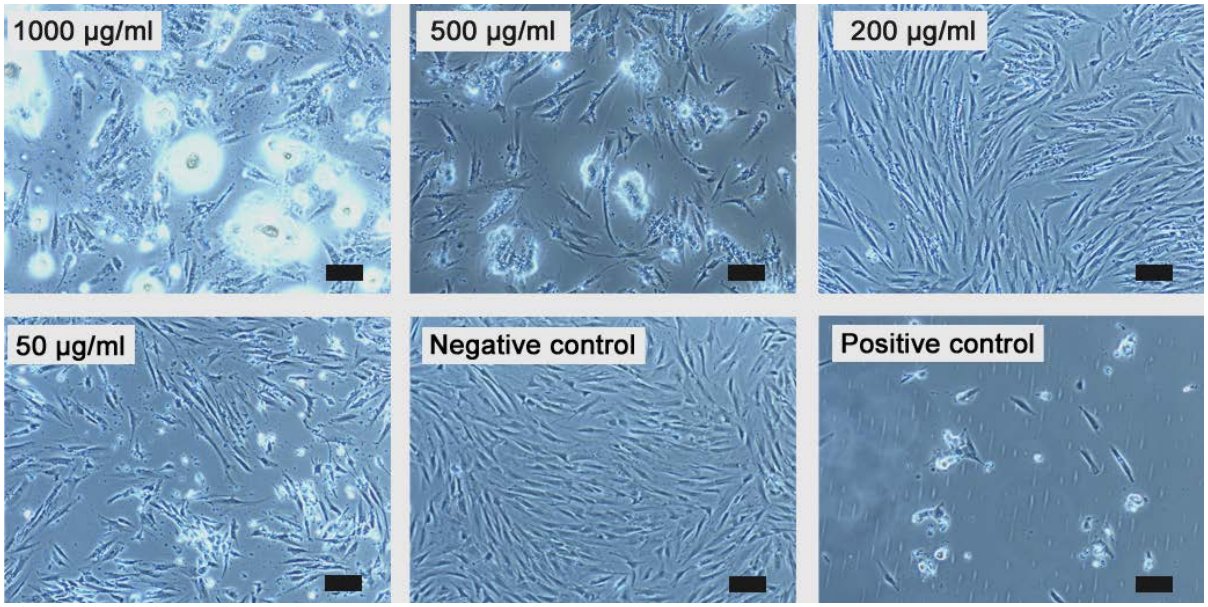

Figure 4. Representative light microscopy images of hDF cells exposed to SBA-15 particle suspensions for 48 hours together with the positive control (5% DMSO) and the negative control (cell culture medium). Scale bars 100 µm.

reactions erythema and edema, see **Table 2**, indicated that the cutaneous reaction was negligible after Upsalite® particle application under the conditions of the study.

3.4. *In Vivo* Acute Systemic Toxicity

All animals appeared clinically normal at the beginning and throughout the *in vivo* acute systemic toxicity study, and there was no mortality. **Table 3** summarizes body weight data for the treatment and control groups. The animal body weight for the 0.9% NaCl extract groups increased and there was no significant difference between the treatment and control group. For the Upsalite® sesame oil extract group significant body weight losses were observed. All mice of this group showed body weight losses 24 hours after injection (between 9.1% and 14.1% body weight loss). However, 72 hours after injection all the mice had recovered their initial body weight. Consequently, there was no evidence of systemic toxicity or mortality after the 0.9% NaCl injection while there was evidence of significant systematic toxicity after the sesame oil extract injection.

The Upsalite® sesame oil extract appeared cloudy with many small and large white particulates present that caused the needles to clog and therefore there were several attempts to inject the mice before succeeding. This is in contrast to the control and 0.9% NaCl extracts where no particulates were observed and hence the needles did not clog. The differences observed between the non-polar and polar extracts were most likely due to the different viscosity of the extracts, *i.e.* in the saline solution Upsalite® particles quickly sediment and therefore the injected extract is free of large particulates, while in the oil extract the particles remain in suspension, clogging the needles and most probably a number of them were injected in the mice.

In order to identify possible leachables, the sesame oil extract was analyzed using ATR-FTIR. The extract spectrum of the sesame oil after extraction appeared identical to the spectrum of the as-received sesame oil.

4. Discussion

In this study the mesoporous magnesium carbonate Upsalite® was the subject of toxicological evaluation by *in vitro* cytotoxicity tests, skin irritation and acute systemic toxicity *in vivo* studies. The material has recently been suggested as drug delivery vehicle [6] [32] and found to have bacteriostatic properties in topical formulations (unpublished results). In order to continue exploring these and other bioapplications of the mesoporous material, primary biocompatibility studies are needed. Herein, we presented a first assessment of the material's toxicity.

For the *in vitro* cytotoxicity tests, fibroblast cells were chosen as model cells. When hDF were directly exposed to Upsalite® suspensions no signs of toxicity were detected: cell viability was above the 70% toxicity limit

Table 2. Dermal observations for the treatment and control groups after Upsalite® particle applications.

		Dermal observations[a]			
Observations*	**Sample**	**1 h**	**24 h**	**48 h**	**72 h**
Erythema	Upsalite®	0	0	0	0
Edema	Upsalite®	0	0	0	0
Erythema	Gauze (control)	0	0	0	0
Edema	Gauze (control)	0	0	0	0

[a]Three animals were used in the study with 2 sites/animal, all animals and sites gave the value 0 for all time points. *0 stands for no signs of erythema or edema.

Table 3. Body weight data for the treatment and control groups of mice after Upsalite® particle extracts injection.

		Weight[a] (g)				
Sample	**Extraction route[c]**	**T0**	**24 h**	**48 h**	**72 h**	**Dead/Tested**
Upsalite®	0.9% NaCl	19.0 ± 0.6	19.4 ± 0.6	21.0 ± 0.5	22.3 ± 0.6	0/5
Control	0.9% NaCl	18.3 ± 0.8	19.0 ± 0.8	19.9 ± 0.9	21.0 ± 0.9	0/5
Upsalite®[b]	Sesame oil	19.1 ± 0.5	17.0 ± 0.4	18.8 ± 0.3	20.6 ± 0.5	0/5
Control	Sesame oil	17.9 ± 0.3	18.6 ± 0.4	19.9 ± 0.4	21.2 ± 0.6	0/5

[a]Five animals were used in the study and injected via the IP route, the data represent the mean weight from all the animals ± standard deviation. [b]The sesame oil extract had to be injected several times due to the presence of particulates (clogged needles). [c]The dose for the extraction vehicle was 50 ml/kg.

for all the tested particle concentrations (**Figure 2(a)** and **Figure 2(b)**) and cells displayed typical fibroblast morphology (**Figure 3**). Interestingly, the reference material mesoporous silica SBA-15 was found to be toxic when tested at 500 - 1000 µg/ml and 48 hours exposure (**Figure 2(d)** and **Figure 4**). In earlier studies with Caco-2 cells SBA-15 particles have been shown to initiate the formation of reactive oxygen species (ROS) already after 3 hours of exposure at a dose of 1000 µg/ml. In that study it was further evident that direct cell-particle interactions were involved in the overall mechanism for cytotoxicity observed from the SBA-15 particles [33]. Since the aim of the present work was not to explore the background for the observed SBA-15 toxicity, the possible formation of ROS by the particles was not studied here. However, it may be speculated that either the formation of ROS by SBA-15 particles or the direct cell-particle interaction (or both) are responsible for the cytotoxicity observed in the present work as well. These results suggest that Upsalite® particles are less toxic for hDF cells than SBA-15 particles at concentrations up to 1000 µg/ml.

The *in vivo* skin irritation study was selected based on very recent findings in our lab showing that Upsalite® can inhibit bacteria growth *in vitro* (unpublished) and that it, hence, may be of interest to evaluate the material as a topical bacteriostatic agent. Encouraging enough, the topical application of Upsalite® did not induce signs of primary skin irritation and the cutaneous reaction was classified as negligible.

In a next step the acute systemic toxicity of Upsalite® particles was studied *in vivo* according to the ISO 10993 part 11 standard [26]. The results from these experiments are less straight forward to interpret, see **Table 3**. For the 0.9% NaCl extractions there were no signs of systemic toxicity, while for the sesame oil extractions the mice presented transient weight loss. The initial weight loss was significant as compared to the control group and therefore the sesame oil extract did not meet the requirements of the ISO 10993-11 standard. At first inspection these results seem to suggest that toxic substances are extracted from the Upsalite® particles with the non-polar extraction medium. However, the sesame oil extract was analyzed with ATR-FTIR and compared with a spectrum of the as-received sesame oil and the two spectra were identical. Of course ATR-FTIR cannot detect all possible extracts but this shows that the sesame oil extract was basically the same as the control injections. Moreover, with the sesame oil extract the mice had to be injected several times due presence of big particulates that clogged the needles. This rather suggests that particles were injected and the toxicity may be caused by the particles themselves (mechanical irritant effect after injection) and not by toxic leachables. This effect has earlier been shown for SBA-15 particles *i.e.* that injecting such particles via the IP route were fatal while injection of

filtrated extraction medium showed no toxicity [9]. The regain of weight observed for the mice further suggests that it was indeed the injection of particles that caused the initial weight loss rather than the effect of toxic leachables.

One way of elucidating the possible role of the particles in the observed systemic toxicity is to include a decantation time or a soft centrifugation of the sesame oil extract before injecting it in the mice. However, the ISO 10993 part 11 guidelines followed in the present study do not contemplate such previous steps and therefore they were not carried out here.

Future research efforts will aim at determining the role of Upsalite® particles in the observed acute systemic toxicity of the non-polar extract.

The results presented here encourage additional investigations of the safety profile of Upsalite®. The focus of such continuation studies will be on application-oriented biocompatibility tests designed to take into consideration the promising application of Upsalite® as drug delivery vehicle [6] [32].

5. Conclusions

The first assessment of toxicity of the mesoporous magnesium carbonate Upsalite® showed that the material was non-toxic for human dermal fibroblasts cells up to a concentration of 1000 µg/ml and 48 h exposure. This was in contrast to the results obtained from the reference material, SBA-15 which significantly affected cell viability after 48 hours exposure at particle concentration of 500 and 1000 µg/ml. It was further found that topical application of Upsalite® resulted in negligible cutaneous reactions. As well, there was no evidence of significant systemic toxicity when saline extracts of Upsalite® were injected in mice. However, the injection of sesame oil extract resulted in transient weight loss in mice. The signs of systemic toxicity observed with the sesame oil extract are most probably an effect of injecting particles, rather than a consequence of toxic leachables.

The results presented here encourage the analysis of potential applications of the mesoporous Upsalite® in the biomedical field. In the future, application-oriented biocompatibility studies will be conducted to further explore the use of Upsalite® as a drug delivery vehicle.

Acknowledgements

The cell studies were performed at the BioMat platform, Science for Life Laboratory, Uppsala University. The Swedish Research Council and the Swedish Energy Agency are gratefully acknowledged for financial support. P. Z. thanks the China Scholarship Council (CSC) for financial support.

References

[1] Lu, G.Q. and Zhao, X.S. (2004) Nanoporous Materials: Science and Engineering. World Scientific.

[2] Fadeel, B., Kasemo, B., Malmsten, M. and Strømme, M. (2010) Nanomedicine: Reshaping Clinical Practice. *Journal of Internal Medicine*, **267**, 2-8. http://dx.doi.org/10.1111/j.1365-2796.2009.02186.x

[3] Vallet-Regí, M. (2010) Nanostructured Mesoporous Silica Matrices in Nanomedicine. *Journal of Internal Medicine*, **267**, 22-43. http://dx.doi.org/10.1111/j.1365-2796.2009.02190.x

[4] Vallhov, H., Kupferschmidt, N., Gabrielsson, S., Paulie, S., Strømme, M., Garcia-Bennett, A.E., *et al*. (2012) Adjuvant Properties of Mesoporous Silica Particles Tune the Development of Effector T Cells. *Small*, **8**, 2116-2124. http://dx.doi.org/10.1002/smll.201102620

[5] Vallhov, H., Gabrielsson, S., Strømme, M., Scheynius, A. and Garcia-Bennett, A.E. (2007) Mesoporous Silica Particles Induce Size Dependent Effects on Human Dendritic Cells. *Nano Letters*, **7**, 3576-3582. http://dx.doi.org/10.1021/nl0714785

[6] Zhang, P., Forsgren, J. and Strømme, M. (2014) Stabilisation of Amorphous Ibuprofen in Upsalite, a Mesoporous Magnesium Carbonate, as an Approach to Increasing the Aqueous Solubility of Poorly Soluble Drugs. International Journal of Pharmaceutics, **472**, 185-191. http://dx.doi.org/10.1016/j.ijpharm.2014.06.025

[7] Strømme, M., Brohede, U., Atluri, R. and Garcia-Bennett, A.E. (2009) Mesoporous Silica-Based Nanomaterials for Drug Delivery: Evaluation of Structural Properties Associated with Release Rate. *Wiley Interdisciplinary Reviews Nanomedicne Nanobiotechnology*, **1**, 140-148.

[8] Brohede, U., Atluri, R., Garcia-Bennett, A.E. and Strømme, M. (2008) Sustained Release from Mesoporous Nanoparticles: Evaluation of Structural Properties Associated with Release Rate. *Current Drug Delivery*, **5**, 177-185. http://dx.doi.org/10.2174/156720108784911686

[9] Hudson, S.P., Padera, R.F., Langer, R. and Kohane, D.S. (2008) The Biocompatibility of Mesoporous Silicates. *Biomaterials*, **29**, 4045-4055. http://dx.doi.org/10.1016/j.biomaterials.2008.07.007

[10] Liong, M., Lu, J., Kovochich, M., Xia, T., Ruehm, S.G., Nel, A.E., *et al*. (2008) Multifunctional Inorganic Nanoparticles for Imaging, Targeting, and Drug Delivery. *ACS Nano*, **2**, 889-896. http://dx.doi.org/10.1021/nn800072t

[11] Peters, K., Unger, R. and Kirckpatrick, C. (2009) Biocompatibility Testing. In: Narayan, R., Ed., *Biomedical Materials*, Springer Science & Business Media, New York, 261-292. http://dx.doi.org/10.1007/978-0-387-84872-3_10

[12] Tang, F., Li, L. and Chen, D. (2012) Mesoporous Silica Nanoparticles: Synthesis, Biocompatibility and Drug Delivery. *Advanced Materials*, **24**, 1504-1534. http://dx.doi.org/10.1002/adma.201104763

[13] Vallet-Regí, M., Balas, F. and Arcos, D. (2007) Mesoporous Materials for Drug Delivery. *Angewandte Chemie International Edition*, **46**, 7548-7558. http://dx.doi.org/10.1002/anie.200604488

[14] Kresge, C.T., Leonowicz, M.E., Roth, W.J., Vartuli, J.C. and Beck, J.S. (1992) Ordered Mesoporous Molecular Sieves Synthesized by a Liquid-Crystal Template Mechanism. *Nature*, **359**, 710-712. http://dx.doi.org/10.1038/359710a0

[15] Beck, J.S., Vartuli, J.C., Roth, W.J., Leonowicz, M.E., Kresge, C.T., Schmitt, K.D., *et al*. (1992) A New Family of Mesoporous Molecular Sieves Prepared with Liquid Crystal Templates. *Journal of the American Chemical Society*, **114**, 10834-10843. http://dx.doi.org/10.1021/ja00053a020

[16] Wan, Y. and Zhao, D. (2007) On the Controllable Soft-Templating Approach to Mesoporous Silicates. *Chemical Reviews*, **107**, 2821-2860. http://dx.doi.org/10.1021/cr068020s

[17] Garcia-Bennett, A.E. (2011) Synthesis, Toxicology and Potential of Ordered Mesoporous Materials in Nanomedicine. *Nanomedicine*, **6**, 867-877. http://dx.doi.org/10.2217/nnm.11.82

[18] Baù, L., Bártová, B., Arduini, M. and Mancin, F. (2009) Surfactant-Free Synthesis of Mesoporous and Hollow Silica Nanoparticles with an Inorganic Template. *Chemical Communications*, **28**, 7584-7586. http://dx.doi.org/10.1039/b917561j

[19] Atluri, R., Hedin, N. and Garcia-Bennett, A.E. (2009) Nonsurfactant Supramolecular Synthesis of Ordered Mesoporous Silica. *Journal of the American Chemical Society*, **131**, 3189-3191. http://dx.doi.org/10.1021/ja8096477

[20] Forsgren, J., Frykstrand, S., Grandfield, K., Mihranyan, A. and Strømme, M. (2013) A Template-Free, Ultra-Adsorbing, High Surface Area Carbonate Nanostructure. *PLoS ONE*, **8**, e68486. http://dx.doi.org/10.1371/journal.pone.0068486

[21] Frykstrand, S., Forsgren, J., Mihranyan, A. and Strømme, M. (2014) On the Pore Forming Mechanism of Upsalite, a Micro- and Mesoporous Magnesium Carbonate. *Microporous and Mesoporous Materials*, **190**, 99-104. http://dx.doi.org/10.1016/j.micromeso.2013.12.011

[22] US Food and Drug Administration. U.S. Department of Health & Human Services.

[23] WHO Department of Essential Medicines and Health Products (2014) The International Pharmacopedia. Fourth Edition, WHO, Geneva.

[24] Brunauer, S., Emmett, P.H. and Teller, E. (1938) Adsorption of Gases in Multimolecular Layers. *Journal of the American Chemical Society*, **60**, 309-319. http://dx.doi.org/10.1021/ja01269a023

[25] ISO 10993-10 (2010) Biological Evaluation of Medical Devices. Part 10: Tests for Irritation and Skin Sensitization.

[26] ISO 10993-11 (2010) Biological Evaluation of Medical Devices. Part 11: Tests for Systemic Toxicity.

[27] Temenoff, J.S. and Mikos, A.G. (2008) Biomaterials: The Intersection of Biology and Materials Science. Prentice Hall, Upper Saddle River.

[28] Zhou, Z., Zhu, S. and Zhang, D. (2007) Grafting of Thermo-Responsive Polymer inside Mesoporous Silica with Large Pore Size Using ATRP and Investigation of Its Use in Drug Release. *Journal of Materials Chemistry*, **17**, 2428-2433. http://dx.doi.org/10.1039/b618834f

[29] Yang, Q., Wang, S., Fan, P., Wang, L., Di, Y., Lin, K., *et al.* (2005) pH-Responsive Carrier System Based on Carboxylic Acid Modified Mesoporous Silica and Polyelectrolyte for Drug Delivery. *Chemistry of Materials*, **17**, 5999-6003. http://dx.doi.org/10.1021/cm051198v

[30] López, T., Basaldella, E., Ojeda, M., Manjarrez, J. and Alexander-Katz, R. (2006) Encapsulation of Valproic Acid and Sodic Phenytoin in Ordered Mesoporous SiO_2 Solids for the Treatment of Temporal Lobe Epilepsy. *Optical Materials*, **29**, 75-81. http://dx.doi.org/10.1016/j.optmat.2006.03.017

[31] ISO 10993-5 (2009) Biological Evaluation of Medical Devices. Part 5: Tests for *in Vitro* Cytotoxicity.

[32] Zhang, P., de la Torre, T.Z.G., Forsgren, J., Bergström, C. and Strømme, M. (2015) Diffusion-Controlled Drug Release from the Mesoporous Magnesium Carbonate Upsalite®. *Journal of Pharmaceutical Sciences*, In Press. http://dx.doi.org/10.1002/jps.24553

[33] Heikkilä, T., Santos, H.A., Kumar, N., Murzin, D.Y., Salonen, J., Laaksonen, T., *et al.* (2010) Cytotoxicity Study of Ordered Mesoporous Silica MCM-41 and SBA-15 Microparticles on Caco-2 Cells. *European Journal of Pharmaceutics and Biopharmaceutics*, **74**, 483-494. http://dx.doi.org/10.1016/j.ejpb.2009.12.006