Acta Odontologica Scandinavica. 2014; 72: 618–622

informa healthcare

ORIGINAL ARTICLE

Positive control for cytotoxicity evaluation of dental vinyl polysiloxane impression materials using sodium lauryl sulfate

JAE-SUNG KWON[1], SANG-BAE LEE[1,2], KWANG-MAHN KIM[1,2] & KYOUNG-NAM KIM[1,2]

[1]*Brain Korea 21 PLUS Project, Department and Research Institute of Dental Biomaterials and Bioengineering, and* [2]*Dental Devices Testing & Evaluation Center, Yonsei University College of Dentistry, Seoul, Korea*

Abstract

Objectives. Vinyl polysiloxane (VPS) is elastomeric dental impression material which, despite having very few reports of adverse reactions, has shown high levels of cytotoxicity that is difficult to be interpreted without referencing to the positive control material. Therefore, in this study, positive control VPS was developed using sodium lauryl sulfate (SLS) for the reference of cytotoxicity test. ***Materials and methods.*** The positive control VPS with SLS was formed with a different proportion of SLS (0, 1, 2, 4, 8 and 16 wt%) added to the base. The cytotoxicity test was then carried out using the extractions or dilutions of the extractions from each of the test samples using murine fibroblast cells (L929). ***Results.*** The final product of positive control VPS behaved similar to commercially available VPS; being initially liquid-like and then becoming rubber-like. Ion chromatography showed that the level of SLS released from the product increased as the proportion of added SLS increased, consequently resulting in an increased level of cytotoxicity. Also, the commercially available VPS was less cytotoxic than the positive control VPS with more or equal to 2 wt% of SLS. However, even the VPS with the highest SLS (16 wt%) did not cause oral mucosa irritation during the animal study. ***Conclusions.*** The positive control VPS was successfully produced using SLS, which will be useful in terms of providing references during *in vitro* cytotoxicity testing.

Key Words: *elastomeric impression materials, vinyl polysiloxane, biocompatibility, cytotoxicity, sodium lauryl sulfate*

Introduction

Elastomeric dental impression materials have been used in dentistry for a long time to accurately replicate the geometry of hard and soft tissues in the oral cavity [1,2]. There are four types of commonly used elastomeric impression materials: polysulfide, polyether, condensation silicones and additional silicone (also known as vinyl polysiloxane (VPS)) [3]. Among them, VPS is the most popular choice due to its elastic characteristics and dimensional stability that allows dentists to replicate oral tissue to a high accuracy [4].

Evaluation of biocompatibility is essential when any medical device is to be used on a patient and cytotoxicity testing using the cell culture technique is the simplest and easiest form of biocompatibility evaluation that can be used to screen a large number of dental materials [5,6].

Several studies have been carried out concerning the cytotoxicity of VPS, where results have indicated a high degree of toxicity towards cell cultures compared to the negative control [2,7,8]; this is despite a few reports of adverse reaction caused by VPS during a history of its use [9]. These results were variable according to the type of cells used and the method of *in vitro* test carried out [10]. Also, and more importantly, results on animal studies often contraindicated the results by *in vitro* studies, which means manufacturers often undergo unnecessary additional animal studies for the acceptance of their product [11].

Therefore, in this study, positive control material for VPS was developed using sodium lauryl sulfate (SLS) in order to provide reference during the *in vitro* cytotoxicity testing, which could then be used to correlate the result of various cytotoxicity studies as

Correspondence: Kyoung-Nam Kim, Brain Korea 21 PLUS Project, Department and Research Institute of Dental Biomaterials and Bioengineering, Yonsei University College of Dentistry, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Republic of Korea. Tel: +82 2 2228 3081. Fax: +82 2 364 9961. E-mail: kimkn@yuhs.ac

(Received 8 October 2013; accepted 19 December 2013)

ISSN 0001-6357 print/ISSN 1502-3850 online © 2014 Informa Healthcare
DOI: 10.3109/00016357.2013.879996

well as *in vivo* studies and avoid unnecessary animal tests.

Materials and methods

Production of positive control VPS using SLS

In order to produce the positive control VPS, the base and the catalyst component of VPS was prepared without the filler. The base component was composed of polyvinyl siloxane and polysiloxane, whereas the catalyst component was composed of polyvinyl siloxane with a platinum catalyst. The varying proportion of sodium lauryl sulfate (SLS, Duksan, Korea; 1, 2, 4, 8, 16 wt% to total VPS) was then added to the base component, where the base component without SLS was used as the control. The base and catalyst component were mixed just before the experiment to produce an initial liquid-like mixture followed by a set rubber-like product, which behaved like any other commercially available VPS.

The release of SLS from the positive control VPS

Each positive control VPS that contained SLS was mixed and allowed to set in a glass tube, which was extracted in distilled water for 24 h according to international standards [12]. The amount of sulfate released during the 24-h period was analyzed using ion chromatography (ICS-2000, Dionex, Sunnyvale, CA, USA), to indirectly indicate levels of SLS released from the positive control VPS.

Cytotoxicity test using positive control VPS

The 'test on extract of materials' [13] was carried out as the cytotoxicity test in this study, where each positive control VPS that contained SLS—as well as two commercially available VPS; Aquasil Ultra XLV (Dentsply, York, PA, USA) and Delikit (Happyden, Seoul, Korea), details are shown in Table I—were tested according to international standards [12–14] and previous studies [7,8,15]. Briefly, the positive control VPS and the commercially available VPS were extracted in serum-free media at 37°C for 24 h. Extractions were then either undiluted or diluted with fresh, serum-free media (50%, 25%, 12.5%, 6.25% and 3.125%) and each of the undiluted/diluted extractions was then placed on a confluent mouse fibroblast of L-929 (Korea Cell Line Bank, Seoul, Korea) in a standard 96 well (SPL, Daegu, Korea). The viability of the cells was assessed following the 24 h of exposure using water-soluble tetrazolium (WST, Daeillab, Korea) assay. The results were expressed as the percentage to the cell cultured by exposure to fresh, serum-free media.

Table I. Summary of commercially available materials used for cytotoxicity test.

Name	Code	Type	Batch no.	Manufacturer
Aquasil Ultra XLV	AU	Vinyl polysiloxane Type 3 Light Body	101202	Dentsply, Germany
Delikit	DK	Vinyl polysiloxane Type 3 Light Body	Gd06442	Happyden, Korea

AU, Aquasil Ultra XLV; DK, Delikit.

Oral mucosa irritation test using positive control VPS

The oral mucosa irritation test was carried out by the Dental Materials Testing and Evaluation Center of Kyungpook National University, Korea, under the rules and regulations of animal ethics of the university. The procedure of the test was in accordance with international standards [16] and previous studies [17,18]. Following the acclimatization of the three Syrian hamsters for 1 week, the positive control VPS that contained 16 wt% of SLS was placed in one of the cheek pouches of each Syrian hamster with the other cheek pouch left unexposed, acting as the control. The exposure was carried out for 15 min every hour for 4 h and a histological examination of the Syrian hamsters' oral mucosa was carried out 24 h after final exposure and sacrificing animals.

Statistics

Linear regression analysis was performed using the SPSS PASW 18.0 program (SPSS Inc., Chicago, IL, USA) to determine the correlation between the amount of SLS released and the weight percentage of SLS in the positive control VPS.

Results

The release of SLS from the positive control VPS

The results of ion chromatography, following extraction of each positive control VPS in distilled water for 24 h, is shown in Figure 1. The results show that there was an increasing concentration of sulfate in distilled water as the proportion of SLS was increased in the positive control VPS. Also, the coefficient of determination (r^2 value) was 0.898; this indicated a high level of correlation between sulfate released and the weight percentage of SLS in the positive control VPS.

Cytotoxicity test using positive control VPS

The results of the cytotoxicity test using a positive control VPS and a commercially available VPS are shown in Figure 2. The results show a high level of cytotoxicity for Aquasil Ultra XLV and a comparably low level of cytotoxicity for Delikit in undiluted extraction when compared to the negative control

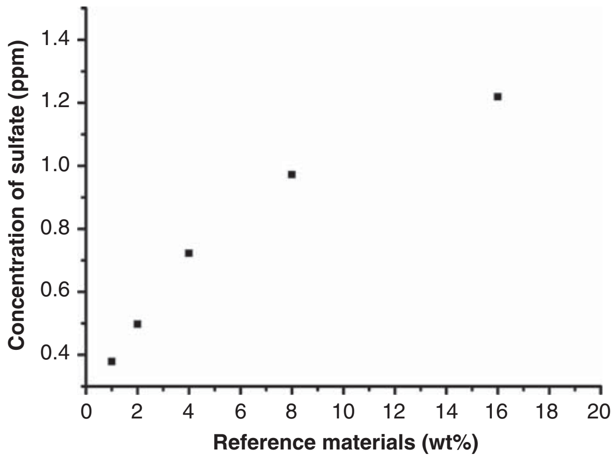

Figure 1. Ion chromatography results for sulfate concentration following extraction of each test materials with different weight percentage of sodium lauryl sulfate (SLS). Coefficient of determination (r^2 value) between concentration of sulfate and weight percentage of SLS in reference material = 0.898.

(Figure 2A), which is similar to previous studies [7]. In terms of the effective concentration of extracts that caused 50% of cell viability compared to the negative control (EC50, dotted horizontal line in Figure 2), Aquasil Ultra XLV was between 25% and 12.5% (Figures 2C and D), whereas Delikit was between 100% and 50% (Figures 2A and B). For the positive control VPS, the results showed that the VPS without SLS (0 wt%) was virtually non-cytotoxic and resulted in near 100% cell viability (Figure 2A), whereas generally increasing cytotoxicity with decreasing the EC50 value was noted as the proportion of SLS was increased. The EC50 of 1 wt% sample was between 100% and 50% of extract concentration (Figures 2A and B), 2 wt% was 12.5% and 6.25% (Figures 2D and E) and all of 4 wt%, 8 wt% and 16 wt% were between 6.25% and 3.125% (Figures 2E and F). Hence it was noted that Aquasil Ultra XLV was more cytotoxic than the 1 wt% sample but less cytotoxic than the 2, 4, 8 and 16 wt% samples in this study.

Oral mucosa irritation test using positive control VPS

The results of the oral mucosa irritation test using a positive control VPS containing 16 wt% of SLS is shown in Figure 3. The results showed that there was no significant difference between the control and the test materials in terms of epithelium, inflammatory cells and vasculature of the oral mucosa during microscopic observation of the histology (Figure 3).

Discussion

Cytotoxicity testing of dental materials is limited in terms of mimicking a real clinical situation due to a lack of inflammatory cells and effects provided by saliva [19]. However, it is still important for the biocompatibility assessment of dental material because it is economical in terms of cost and time required, while also being more ethical compared to animal studies [5,6,19].

Numerous studies were carried out concerning cytotoxicity of elastomeric dental impression materials, especially in relation to VPS [2,7,8,20], which showed a relatively high level of cytotoxicity compared to the negative control. The possible reason for such cytotoxicity was explained by release of a surfactant from the VPS, which was added to improve the

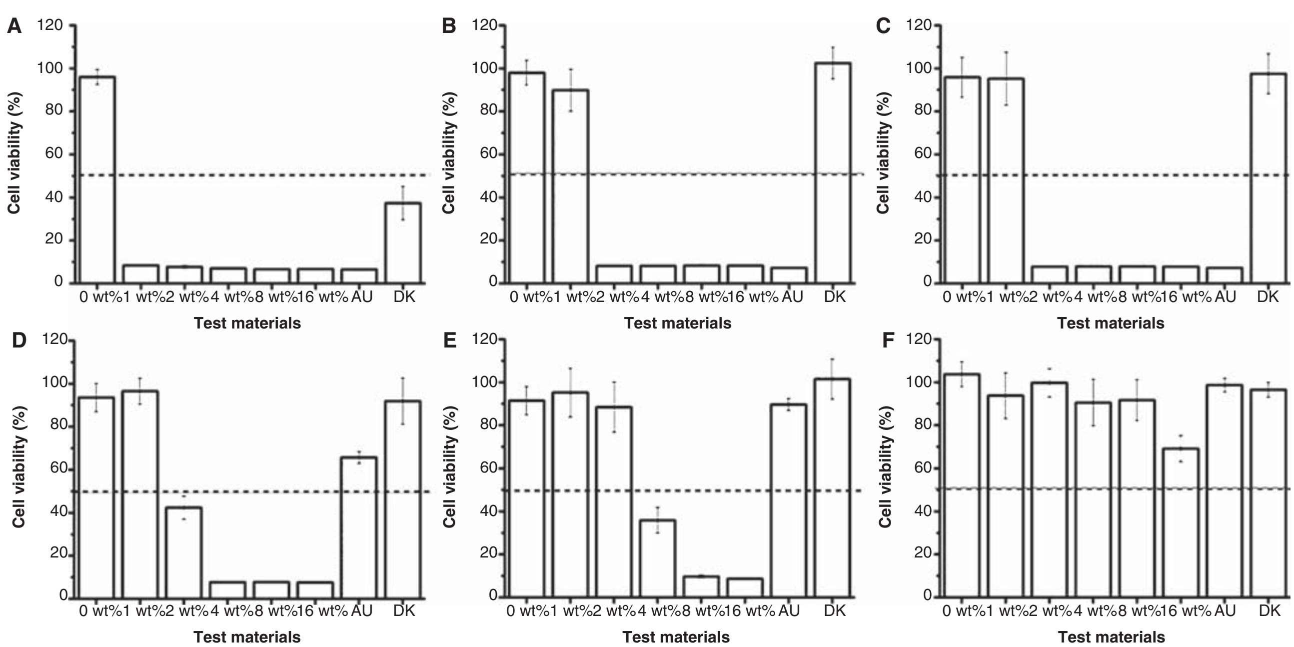

Figure 2. Cytotoxicity test results following exposure to extract of each test and commercially available materials for 24 h. Extracts were diluted to; (A) 100% (i.e. undiluted), (B) 50%, (C) 25%, (D) 12.5%, (E) 6.25% and (F) 3.125%.

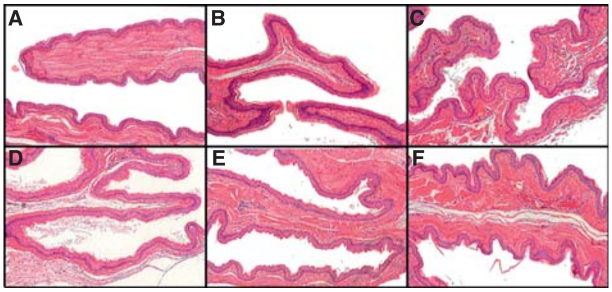

Figure 3. Oral mucosa irritation test results following exposure to reference positive control vinyl polysiloxane with 16 wt% of sodium lauryl sulfate. Control oral mucosa which were not exposed to test materials are (A), (B) and (C), whereas oral mucosa exposed to test materials are (D), (E) and (F). Each pair of top and bottom figure belonged to one Syrian hamster.

hydrophilicity of hydrophobic VPS for the precision of the impression and the die [2,21,22]. However, even though there were few reports of adverse foreign body reactions by the VPS [23,24], especially when pieces of materials were left behind to come in contact with tissue for a long period of time, such cases were very rare considering the length of time VPS has been used in dentistry. Also, most of animal studies have shown no toxic reactions towards such VPS [11], which contraindicated the *in vitro* studies and demand new ways of testing the *in vitro* cytotoxicity test for VPS, in terms of prediction of biological reactions by such materials for both animal and patient studies.

Hence, in this study, the positive control material for VPS was developed using SLS, which could be used to provide reference to the cytotoxicity results as well as the possibility of correlating the *in vitro* results to the *in vivo* results.

Sodium lauryl sulfate is an anionic surfactant that has been commonly used for cleaning agents and dentifrices [18]. It is also commonly used as the reference chemical in cytotoxicity studies and animal toxicology studies due to its known basal cytotoxicity that disrupts the membrane of cells while causing desquamation of oral epithelial cells [25,26].

The initial aim of this study was to produce a positive control VPS that behaved like other commercially-available VPS, while producing different degrees of cytotoxicity according to the proportion of SLS added. In fact, this is also in line with a recently amended international standard for 'Dentistry- Evaluation of biocompatibility of medical devices used in dentistry' [27], which states that positive control materials shall provide a different degree of toxic response while being 'handled and processed like the test materials' [27]. In order to achieve this, base and catalyst components that had similar components to commercially available products such as polyvinyl siloxane, polysiloxane and platinum catalyst were used to produce the material that was initially liquid-like but later became rubber-like when mixed. Also, in order to test the release of SLS that was the main cytotoxic component, ion chromatography was carried out (Figure 1). The results of ion chromatography showed that the release of SLS was proportional to the weight percentage of SLS added with very high correlation ($r^2 = 0.898$). It is well known from previous studies that most of the surfactant in VPS diffuses through the material to reach the most outer layer, which then leaches into surrounding liquid through diffusion [28,29]. Therefore, the increased amount of SLS added in the positive VPS was reflected on the increased amount of sulfate found during the extraction procedure, as detected by ion chromatography analysis.

Such released SLS then affected cell viability, as shown by the cytotoxicity test (Figure 2). Samples without SLS showed virtually no level of cytotoxicity, but VPS with even a small amount of SLS (1 wt%) resulted in a high level of cytotoxicity and increasing proportions of SLS resulted in increasing levels of cytotoxicity, as indicated by the EC50 value. As previously mentioned, SLS is known to affect the phospholipids on the cell membrane while also disrupting the bonding of proteins causing denaturation [25,30] which, therefore, results in cytotoxicity. However, it was interesting to note that the EC50 of the previously mentioned cytotoxic commercial product (Aquasil Ultra XLV; EC50 between 12.5–25%) was higher than the reference positive control VPS with more or equal to 2 wt% SLS (EC50 between 6.25–12.5%). Indeed, Aquasil Ultra XLV was much less cytotoxic than the highest SLS sample used in this study, the reference positive control VPS with 16 wt% SLS.

The reference positive control VPS with 16 wt% SLS was then used to test the oral mucosa irritation of the Syrian hamsters (Figure 3). The results showed that even the highest SLS sample used in this study did not cause any irritation on oral mucosa during the animal studies. Although it is often difficult to correlate *in vitro* studies with *in vivo* studies due to the limitation of mimicking environments as mentioned previously, it was reasonable to conclude from the animal studies that no positive reactions would have been identified with both of the commercially available VPS that were tested in this study.

Therefore, in this study, positive control VPS materials were successfully developed to behave like other commercially available VPS in terms of the process of mixing and setting, using SLS. It produced positive cytotoxicity effects to be considered adequate as the positive control material and variable cytotoxicity according to the proportion of SLS allowed it to be a reference for *in vitro* studies. Also it provided means of correlating the *in vitro* biocompatibility test with *in vivo* studies. However, future studies with the weight percentage of SLS that result in positive reactions toward animal studies, and apply such reference positive control VPS on other forms of *in vitro* cytotoxicity testing, will be useful in terms of providing a reference to tested materials during the *in vitro* test and also the possibility of avoiding animal studies. Despite these limitations, the positive control VPS was successfully produced using SLS, which will be useful in terms of providing reference during *in vitro* cytotoxicity testing of commercially available or newly developed VPS, while possibility avoiding the need for animal studies. Such methods may also be applicable to other forms of dental materials.

Acknowledgments

This research was supported by the Dental Devices Testing & Evaluation Center, Yonsei University College of Dentistry and by a grant (14172MFDS334) from Ministry of Food and Drug Safety.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Brown D, Clarke RL, Curtis RV, Hatton PV, Ireland AJ, McCabe JF, et al. Dental materials: 1994 literature review. J Dent 1996;24:153–84.
[2] Chen SY, Chen CC, Kuo HW. Cytotoxicity of dental impression materials. B Environ Contam Tox 2002;69:350–5.
[3] Brunton PA, Christensen GJ, Cheung SW, Burke FJT, Wilson NHF. Contemporary dental practice in the UK: indirect restorations and fixed prosthodontics. Brit Dent J 2005;198:99–103.
[4] Allen EP, Bayne SC, Brodine AH, Cronin RJ Jr, Donovan TE, Kois JC, et al. Annual review of selected dental literature: report of the committee on scientific investigation of the American Academy of Restorative Dentistry. J Prosthet Dent 2001;86:33–56.
[5] Schmalz G. Concepts in biocompatibility testing of dental restorative materials. Clin Oral Investig 1997;1:154–62.
[6] Williams DF. On the mechanisms of biocompatibility. Biomaterials 2008;29:2941–53.
[7] Kwon JS, Lee SB, Kim CK, Kim KN. Modified cytotoxicity evaluation of elastomeric impression materials while polymerizing with reduced exposure time. Acta Odontol Scand 2012; 70:597–602.
[8] Sydiskis RJ, Gerhardt DE. Cytotoxicity of impression materials. J Prosthet Dent 1993;69:431–5.
[9] Mazzanti G, Daniele C, Tita B, Vitali F, Signore A. Biological evaluation of a polyvinyl siloxane impression material. Dent Mater 2005;21:371–4.
[10] Coppi C, Devincenzi CP, Bortolini S, Consolo U, Tiozzo R. A new generation of sterile and radiopaque impression materials - an *in vitro* cytotoxicity study. J Biomater Appl 2007;22: 83–95.
[11] Browne RM. The *in vitro* assessment of the cytotoxicity of dental materials–does it have a role? Int Endod J 1988;21:50–8.
[12] ISO documents. ISO 10993-12. Biological evaluation of medical devices – Part 12: sample preparation and reference materials. Geneva: International Standard; 2002.
[13] ISO documents. ISO 10993-5. Biological evaluation of medical devices – Part 5: tests for *in vitro* cytotoxicity. Geneva: International Standard; 2009.
[14] ISO documents. ISO 10993-1. Biological evaluation of medical devices – Part 1: evaluation and testing within a risk management process. Geneva: International Standard; 2009.
[15] Kwon JS, Illeperuma RP, Kim J, Kim KM, Kim KN. Cytotoxicity evaluation of zinc oxide-eugenol and non-eugenol cements using different fibroblast cell lines. Acta Odontol Scand 2014;72:64–70
[16] ISO documents. ISO 10993-10. Biological evaluation of medical devices – Part 10: tests for irritation and skin sensitization. Geneva: International Standard; 2010.
[17] Baert JH, Veys RJ, Ampe K, De Boever JA. The effect of sodium lauryl sulphate and triclosan on hamster cheek pouch mucosa. Int J Exp Path 1996;77:73–8.
[18] Veys RJ, Baert JH, De Boever JA. Histological changes in the hamster cheek pouch epithelium induced by topical application of sodium lauryl sulphate. Int J Exp Path 1994;75: 203–9.
[19] Neppelberg E, Costea DE, Vintermyr OK, Johannessen AC. Dual effects of sodium lauryl sulphate on human oral epithelial structure. Exp Dermatol 2007;16:574–9.
[20] Ciapetti G, Granchi D, Stea S, Savarino L, Verri E, Gori A, et al. Cytotoxicity testing of materials with limited *in vivo* exposure is affected by the duration of cell-material contact. J Biomed Mater Res 1998;42:485–90.
[21] Mandikos MN. Polyvinyl siloxane impression materials: an update on clinical use. Australian Dent J 1998;43:428–34.
[22] Rupp F, Axmann D, Jacobi A, Groten M, Geis-Gerstorfer J. Hydrophilicity of elastomeric non-aqueous impression materials during setting. Dent Mater 2005;21:94–102.
[23] Eliasson ST, Holte NO. Rubber-base impression material as a foreign body. Report of a case. Oral Surg Oral Med Oral Pathol 1979;48:379–80.
[24] Sivers JE, Johnson GK. Adverse soft tissue response to impression procedures: report of case. J Am Dent Assoc 1988;116:58–60.
[25] Bednarkiewicz A, Rodrigues RM, Whelan MP. Non-invasive monitoring of cytotoxicity based on kinetic changes of cellular autofluorescence. Toxicol In Vitro 2011;25:2088–94.
[26] Kowitz G, Lucatorto F, Bennett W. Effects of dentifrices on soft tissues of the oral cavity. J Oral Med 1973;28:105–9.
[27] ISO documents. ISO 7405, Amd 1. Dentistry-Evaluation of biocompatibility of medical devices used in dentistry, Amd 1: positive control materials. Geneva: International Standard; 2013.
[28] Grundke K, Michel S, Knispel G, Grundler A. Wettability of silicone and polyether impression materials: characterization by surface tension and contact angle measurements. Colloid Surface A 2008;317:598–609.
[29] Kanehira M, Finger WJ, Komatsu M. Surface detail reproduction with new elastomeric dental impression materials. Quintessence Int 2007;38:479–88.
[30] Repetto G, del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat Protoc 2008;3: 1125–31.