Novel polylactic acid (PLA)-organoclay nanocomposite bio-packaging for the cosmetic industry; migration studies and in vitro assessment of the dermal toxicity of migration extracts

Novel polylactic acid (PLA)-organoclay nanocomposite bio-packaging for the cosmetic industry; migration studies and *in vitro* assessment of the dermal toxicity of migration extracts.

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Abbreviations[1]

[1] Å: Armstrongs
CAS no: Chemical abstracts service number
dm^2: square decimeter
DMEM: Dulbecco's Modified Eagle's Medium
GPa: gigapascals
HaCaT: immortal human keratinocyte cell line
HDTA: Hexadecyl Trimethyl ammonium bromide
ISO: International Organization for Standardization
MEM minimal essential medium
OECD TG: Organisation for Economic Co-operation and Development Test Guideline
PLA: polylactide
TOM: Total overall migration
TMSA: octadecyl trimethyl ammonium chloride
wt/wt: weight per weight
XRD: X-ray diffraction

Abstract

The exploitation of polylactide (PLA) nanocomposites (which integrate organically modified clays (organoclays) into polymers) in packaging for the cosmetics industry could provide a biodegradable alternative to the use of conventional plastics. In this study nanocomposites with a PLA polymer matrix and clay fillers organically modified with the quaternary ammonium salts, hexadecyl trimethyl ammonium bromide (HDTA) or octadecyl trimethyl ammonium chloride (TMSA), were produced and tested for their safe use in cosmetic packaging. To address concerns over the potential release of constituents from such nanocomposites, levels of total overall migration in a range of simulants (e.g. vegetable oil, aqueous media, and cosmetic formulations) was assessed 10 days post incubation at 40 ˚C following EU-Plastics Regulation 10/2011 concerning materials and articles in contact with foodstuffs. Total overall migration levels calculated for all PLA nanocomposites tested (maximum of 0.88 ±0.44 mg/dm^2) were well below the total established legislative migration limit (10 mg/dm^2). Toxicity of the nanocomposite migration extracts to the skin was assessed *in vitro*. Exposure of skin cells (HaCaT immortalized human keratinocytes) and a full thickness epidermal skin model (EpiDerm^{TM}) to migration extracts did not result in any significant loss in cell viability or skin irritation (OECD TG 439). The results therefore indicate that the levels of migration from the nanocomposite measured was low, and that the nanocomposite migration extracts stimulated minimal toxicity to the skin. Up until now, the hazard of migration extracts from polymer-organoclay nanocomposites following dermal exposure has not been investigated and thus our study addresses a gap in knowledge. These findings can inform the safe design of bio-based biodegradable nanocomposite packaging (used by the cosmetics, and other industries) in the future to promote a more sustainable and greener economy for the plastics industry.

Keywords: PLA nanocomposites; organoclays; migration; packaging; dermal exposure; skin irritation

1. Introduction

Clays composed of nanoscale layered aluminosilicate particles (1 nm thick, 200-300 nm long) are being increasingly used as fillers within polymers to produce next generation high performance materials known as nanocomposites [1] (Paul and Roberson 2008). Packaging represents nearly half (39.9 %) of the total plastic demand in Europe [2] (Plastics Europe 2017) and replacing petroleum-based plastics with such bio-based nanocomposites represents a giant leap towards a more sustainable and greener economy. For example, bio-based polymer-clay nanocomposites are being investigated as alternative, more environmentally-friendly, materials to petroleum-based plastics [3, 4] Reedy et al. 2013, Ray et al. 2006). One of the most promising and widely used bio-based polymers, that has already been commercialised, is polylactide (PLA). PLA is made from the polymerisation of lactic acid monomers that are produced through the fermentation of sugars which have been extracted from a biomass source (e.g. corn starch, tapioca, sugar cane, or sugar beet)[5](John et al. 2007). PLA is not only bio-based but compostable and biodegradable through hydrolysis by micro-organisms [6](Ghorpade et al., 2001). It is a thermoplastic polymer with high processability, its production capabilities are currently at an industrial scale (150,000 (metric) tonnes per annum (NatureWorks ® LLC. USA)) and it has a forecasted market annual growth rate of 17.2% from 2018 to 2023 [7](Reports Database 2018). Yet currently it represents only ~1% of the total plastics produced globally [8](European Bioplasics, 2017). Slowing down its widespread use up until now is cost competitiveness with conventional polymers, aswell as its brittleness and poor gas barrier properties. To address one of these limitations nanoscale structured layered clay particles can be incorporated into PLA conferring strength, increasing its gas barrier properties [9,10,11](Ray et al. 2002; Prakalathan et al. 2012; Darie et al. 2014), and ultimately allowing the nanocomposite to compete with conventional plastics in a wide range of applications [12](Murariu and Dubois 2016). Indeed, an improvement in mechanical and barrier properties of PLA-organoclay nanocomposites compared to PLA polymers alone has already been demonstrated previously [13,14](Jorda-Beneyto et al. 2014, Chang et al. 2003).

Packaging performance has often been reported to be influenced by the dispersion state and structure of the clays in the nanocomposite [15,16](Rhim et al. 2009, Sengul et al. 2004). For this reason, clays are often organically modified by replacing the interlayer cations of the clay sheets with quaternary ammonium or phosphonium cations to make them less hydrophilic and more compatible with the polymer matrix [17,18](Maiti et al. 2002, Ray et al. 2003). Depending on the degree of dispersion of the nanostructured clay in the polymer nanocomposite a phase separated (clay platelets in large stacks), intercalated (increased inter-layer spacing of clay platelets) or exfoliated (individual platelets distributed throughout the PLA matrix) nanocomposite will result [19,2](Alexandre and Dubois, 2000, Dennis et al. 2001). Improved performance with exfoliated polymer organoclay nanocomposites has been shown [21](LeBaron et al. 1999). Over 70% of all PLA produced is used in the packaging industry [22](Siracusa et al. 2008) and organoclays are the most utilised nanofillers [23](Marquis et al. 2011).

Existing research has focused primarily on the development of bio-packaging for the food industry [24,25,26](Youssef 2013; Rhim et al. 2013; de Azeredo 2009) and applications for short shelf-life fresh products. However, there also exists a market within the cosmetic packaging industry which relies heavily on plastic for packaging producing over 76.8 billion plastic packaging units each year (Euromonitor, 2017). The development of a bio-based and biodegradable packaging alternative, particularily for organic or eco-friendly lines would offer complete product differentiation and a solution to the global plastic waste problem.

However, ensuring the safe use of bio-based nanocomposites in the packaging industry must not be overlooked. Already in conventional polymer plastics, such as polyvinyl chloride (PVC) and polyethylene terephthalate (PET), the release of substances such as bisphenol A (BPA) and certain phthalates has raised concerns surrounding the safety of packaging [27,28,29](Boˇsnir et al 2007, Bach et al. 2012, Fasano et al. 2012). These chemicals may have endocrine disrupting properties, and they can be absorbed through the skin [30](Zalko et al. 2011) and so, at present, restrictions and

specific migration limits on BPA use in food contact materials have been set to protect consumers [31](EU 2018). On a regulatory level, clays (including bentonite) are approved as additives in bulk form for use in cosmetic and personal care products. However, when used in polymer nanocomposites clays may be present in an exfoliated, deagglomerated state and thus any hazard associated with exposure through potential migration of individual clay sheets from nanocomposite packaging into cosmetic formulation needs to be assessed to ensure their safe use. Individual alumino-silicate sheets are truly nanoparticulate (~1nm in thickness), with a high aspect ratio (ranging from 200–500:1) [32](Uddin 2018). Safety concerns surrounding nanomaterial sheets (e.g graphene nanosheets)[33](Xu et al., 2015) and high aspect ratio fibres have been raised previously [34,35](Donaldson et al. 2010, Xia et al. 2016). Studies on dermal exposure route are lacking.

Furthermore, the use of organically modified clays in nanocomposites may be of concern due to the potential for the chemical modifier to enhance the toxicity of the clays [36,37,38,39](Janer et al. 2014, Lordan et al. 2011, Wagner et al. 2017, Connolly et al. 2019) or migrate from the packaging material into the product [40](Xia et al. 2014). Commonly used chemical modifiers belong to a group of quaternary ammonium compounds that are used as cationic disinfectants and biocidal agents to prevent the growth of microorganisms [41](Gerba et al. 2015). They are approved for use in cosmetics and personal care products as preserving agents and detergents, albeit with specific concentration limits due to their inherent skin irritating properties [42](Lin and Hemming 1996). They also have shown cytotoxic and genotoxic effects towards several human cells *in vitro*, including HaCaT skin cells, C3A hepatocytes and J774.1 macrophage-like cells [43,44,39](Nagamune et al. 2000, Ferk et al. 2007, Connolly et al. 2019).

At present information is lacking on the potential of clays/organoclays to migrate from polymer nanocomposites [45](Jokar et al. 2017). The extent to which these materials will cause adverse effects if released from packaging is also not clear. A limited number of migration studies have been performed, mainly focusing on nanocomposites used in food contact materials in the food packaging

industry [46,40,47](Echegoyen et al. 2016, Xia et al. 2014, Huang et al. 2015), where oral exposure will result from release/migration. This study seeks to investigate the safety of PLA polymer-organoclay nanocomposites used in the cosmetic packaging industry, where migration will result in dermal exposure. To the best of our knowledge, the hazard of polymer organoclay nanocomposite migration constituents, following dermal exposure, has not been studied previously or reported on. Within the cosmetic industry there are no specific guidelines concerning packaging/content interaction studies. Yet, according to the EU Cosmetic Regulation 1223/2009 there is a requirement to report on the characteristics of packaging materials, such as stability, impurities, and traces, within a product's safety assessment. The exposure of skin to non-intended substances stemming from migration from packaging must be at a level that is safe for human health (Annex I, Regulation (EC) No 1223/2009)[48](EC 2009). Further challenges in the safety assessment of such packaging material arise for the need to assess hazard using alternatives to animals, due to the ban on animal testing imposed on the cosmetic industry for products and ingredients marketed in the EU [48](EC 2009). Other specific considerations include the use of solvents and other ingredients in cosmetic formulations (e.g. paraffin, glycerine, propylene glycol, and ethyl alcohol) that may promote the degradation of the polymer and increase the migration potential of (hazardous) packaging components into the product [49](Rydz et al. 2013). For example, PLA is readily degradable through hydrolysis and may be susceptible to breakdown in strong acidic or basic pH environments or solvent induced crystallisation [50](Iñiguez-Franco et al. 2016).

Within this study bio-based and biodegradable PLA polymer-organoclay nanocomposites were produced and tested for their safe use in cosmetic packaging. The biocompatibility of the PLA polymer-organoclay nanocomposites, the levels of migration of components from the nanocomposite, and potential for migration extracts to cause adverse effects was investigated. Migration studies were performed according to guidelines for food contact materials [51](EC 2011) using vegetable oil, and using cosmetic formulations as simulants to mimic real use scenario

conditions. Human HaCaT keratinocyte skin cells were used to test adverse effects from exposure of the skin *in vitro* to migration extracts via assessment of cytotoxicity. In addition, a reconstructed 3D human skin model (EpiDerm^{TM}) was used to assess the skin irritation potential of migration extracts, according to OECD TG 439. Four types of PLA based nanocomposites were produced, using different grades of PLA and processing techniques (injection moulding and extrusion) and incorporating two different organoclays modified with hexadecyl trimethyl ammonium bromide (HDTA) or octadecyl trimethyl ammonium chloride (TMSA).

2. Materials and Methods

2.1 Production of PLA polymer-organoclay nanocomposites

Nanocomposites were produced using two different grades of PLA: Ingeo™ Biopolymer 2003D (PLA1) and Ingeo™ Biopolymer 3052D (PLA2) from NatureWorks LLC. The PLA305D was selected due to its applicability for injection moulding, while PLA2003D is suitable for processing on conventional extrusion equipment. Sodium activated bentonite clays (Nanofil®116, BYK Additives and Instruments, USA) (CAS no: 1302-78-9)) were organically modified in house as described by [13]Jorda Beneyto et al. (2014) and [39] Connolly et al. (2019) with the quaternary ammonium compounds hexadecyl trimethyl ammonium bromide (HDTA) (CAS no. 57-09-0) (27.3 g/100 g clay) or octadecyl trimethyl ammonium chloride (TMSA) (CAS no. 112-03-8) (29.5 g/100 g clay) (CymitQuimica S.L., Spain). Nanocomposites were prepared using organoclay loading of 4 % (wt/wt). A plasticiser was also added (20 % wt/wt) to PLA1 to enhance flexibility and improve processing.

Nanocomposite samples were prepared by different techniques at laboratory scale, depending on the PLA grade. For PLA1 nanocomposites, extruded sheets with a thickness of 400 µm were obtained in a twin-screw extrusion line with a die head for film, while for PLA2 nanocomposites, circular specimens with a thickness of 800 µm were obtained by micro-compounding with an injection system. Processing conditions were followed according to the technical datasheets from polymer

suppliers (NatureWorks LLC, USA).Discs (13 mm in diameter) were cut from sheets of each nanocomposite using an Epilog Mini 40W Laser Cutter at 60% Power, 100% Speed and 5kHz Frequency (see supplementary figureS1 for photographs of polymer nanocomposites and discs). These discs were sterilised in a laminar flow cabinet using UV light (40 min), soaking in an antibiotic solution overnight (1% Penicillin/Streptomycin mixture prepared in sterile water), followed by dipping in 80% ethanol and a rinse in sterile water. These nanocomposite samples were named according to the PLA grade and the organic modifier used; i.e. PLA1_HDTA, PLA1_TMSA, PLA2_HDTA and PLA2_TMSA.

2.2 Characterisation of PLA-organoclay nanocomposites

Wide-angle X-ray diffraction (XRD) (D8 Advance A25 (Bruker)) was used to investigate the structure of the clays within the PLA nanocomposites as a measure of dispersion state. Crystalline phases present in the PLA nanocomposites with unmodified parent clays (N116) and organoclays were analysed. The influence of different organic modifier levels (e.g. 2, 4, 6 and 8 times the theoretical amount of modifier required to exchange all the available cations from the clay) on organoclays dispersion state within nanocomposites was also investigated.

The interlayer d-spacing (the distance between the basal layers of the clay) was calculated from the XRD spectrum according to the 2Θ angles corresponding to the diffraction peaks of the organoclays by the Bragg's Law, whose formula is:

$n\lambda = 2d \sin\theta$

Where, n is the order of diffraction, λ is the wave length, θ is the angle of diffraction and d- inter planar distance. According to Bragg's law; the d-spacing is inversely proportional to the scattering angle. Therefore the shift of the peaks to lower scattering angles corresponds to larger d-spacing and hence larger distances between the silicate layers will be found. Samples were also viewed under light microscopy (40 X magnification).

Mechanical properties (Young´s modulus and elongation at break) of the two pure PLA grades as well as PLA nanocomposites were evaluated using a universal testing machine (model M350-20CT), following standard ISO-527.

2.3 In vitro biocompatibility studies

A direct contact test method was used to evaluate the acute adverse biological effects of migration extracts from the PLA polymer-organoclay nanocomposites (adapted from ISO 10993-5: 2009 for testing medical devices) [52](ISO 2009). According to this method cells are grown in direct contact with the test article in an *in vitro* test system and any effect on cell growth, proliferation, or a reduction in viability following direct contact with the test material is measured.

Sterile nanocomposite discs (13 mm diameter, either 400 μm (PLA1) or 800 μm thick (PLA2)) were placed in the bottom of 24-well microplates (Coaster Corning Flintshire, UK). PLA1 and PLA2 discs without organoclays but with the same respective thickness served as controls. Cell culture polyester coverslips (13 mm diameter, 200 μm thick) (Coaster Corning, Flintshire, UK) were used as a reference to compare cell attachment and proliferation with the nanocomposite discs.

Epidermal human keratinocytes (HaCaT cells) (CLS cell lines service, Eppelheim, Germany) were seeded (2.5 x10^5 cells/mL, 500μl/ well) directly onto the sterilised discs and coverslips or in well and incubated for 24 or 48h at 37 ˚C/5% CO_2 in DMEM culture medium with a high glucose content (4.5 g/L) (Thermo Fisher Scientific, Cramlington, UK) and supplemented with 10% heat-inactivated foetal bovine serum (FBS) (Thermo Fisher Scientific, Cramlington, UK), 100 U/ml Penicillin and 100 μg/ml Streptomycin (Gibco, USA) and 2mM L-glutamine (Gibco® ThermoFischer Scientific, UK)) (termed complete MEM).

After 24 h or 48 h cells were observed (optical microscopy) for visible signs of adverse effects (such as a change in the size or appearance of cells or a disruption in their growth pattern) in response to

the test and control materials. Neutral red dye (Sigma Aldrich, UK) staining (0.03 µg/mL) was used to aid in visualising the cells. Levels of viability were assessed using the alamarBlue® assay which assesses viability based on the metabolic activity of cells. The conversion of alamarBlue® (ThermoFisher Scientific, UK) (1.25% (v/v)) following 1h incubation (37°C/5 % CO_2) to the fluorescent product resorufin was measured at excitation/emission wavelengths of 560 nm and 590 nm to assess the level of viability of cells. Data are expressed as a percentage of the levels of viability measured in control cells grown directly in culture plate.

2.4 Migration studies

Migration tests for total overall migration were performed using test methods that have been standardized according to British adopted European standard BS EN 1186-1:2002 [53](BS EN 2002), and guidelines set out in the EU-Plastics Regulation 10/2011 concerning materials and articles in contact with foodstuffs [51]EC 2011). PLA and nanocomposite discs were weighed and placed into sterile 7 mL glass bijou vials (Samco, SLS, UK) which acted as migration cells. Two different cosmetic formulations (Cosmetic A and B) were used as simulants to mimic specific use conditions in the cosmetic packaging industry. Cosmetic A was supplied by Alan Coar, Spain and represented a simple oil in water emulsion. Cosmetic B was supplied by Alissi Bronte, Spain and represented a highly lipophilic emulsion. Vegetable oil (Simulant D) was selected for use as it used as a reference simulant for fatty foods according to Plastic regulation (EU) 10/2011 [51](EC 2011) and most closely resembles the cosmetic formulations being used. Samples were also tested in contact with aqueous based biological culture medium (minimal essential medium) (MEM) (Fisher Scientific, UK) according to MEM elution assay guidelines for cytotoxicity testing (ISO 10993-5) to generate samples that could be used to assess the response of HaCaT cells to potential migrants. Samples were tested in each simulant in quadruplicate. Cell culture polyester coverslips (13 mm in diameter and 200 µm thick) served as control samples, and blanks (simulants only) were also included. Discs were placed flat at the bottom of the migration cell and simulants (0.472 mL) were added directly on top creating

a single surface testing setup. The contact surface area was 132.65 mm^2 (or 1.3265 dm^2). This contact surface area to simulant volume ratio mimicked conditions of intended use of the nanocomposites as a cosmetic packaging material (see supplementary figure S2). Migration cells were kept at 40 ˚C for 10 days in reduced light according to test migration conditions outlined for long term storage ((EU) Regulation No 10/2011).

At the end of the test period, discs were removed, washed with sterile deionised water to remove any cosmetic/simulant remaining on the surface, and allowed to dry overnight at room temperature. The weight of each disc was then recorded and compared to weights measured before the test began to calculate total overall migration. Total overall migration (M) was calculated using the formula below and expressed as milligrams (mg) per decimetre (dm) of the surface of the sample.

$$\text{Overall migration: } M\ (mg/dm^2) = \frac{(W2-W1)\ (mg)}{S\ (dm^2)}$$

where:

W1 is the weight of the disc prior to testing, in milligrams.

W2 is the weight of the disc after the contact period, in milligrams.

S is the surface area of the test specimen intended to come into contact with simulant, in square decimetres.

2.5 Hazard assessment of migration extracts

2.5.1 MEM elution assay

The cytotoxicity of migration extracts towards skin cells was assessed using the MEM elution test which was developed as part of the ISO standard series of tests for *in vitro* cytotoxicity for biological evaluation of medical devices (ISO-10993-5) [52](IS0 2009).At present, there is no specific test

guideline or procedural document for testing the hazard of migration extracts to the skin. Therefore the above standardised test procedure was selected and adapted for use with HaCaT skin cells.

Minimal essential medium (MEM) simulants that were in contact with PLA and PLA nanocomposites were collected from migration cells and tested for cytotoxicity *in vitro* using HaCaT skin cells. Cells were seeded at a density 2.5 x10^5 cells/mL (100µl) in 96 well microplates and incubated for 24h at 37 ˚C/5% CO_2 atmosphere. HaCaT cells were then exposed to the MEM simulants (100µl) that were held in contact with the PLA polymers and nanocomposites and tested in triplicate. MEM simulants collected from migration cells with plastic coverslip and blanks served as controls. Following 24 h exposure, cells were washed with phosphate buffered saline and then incubated with a 1.25% (v/v) alamarBlue® solution prepared in complete MEM for 1 h (37 °C/5% CO_2). Fluorescence was then measured at excitation/emission wavelengths of 560 /590 nm. Data are expressed as a percentage of the levels of viability measured in control cells (cells in exposed to complete MEM).

2.5.2 Skin irritation testing

Skin irritation tests were performed using the reconstructed human epidermal tissue model, EpiDerm™ SIT (EPI-200) (MatTek, Slovakia) (Lot no: 23324, Kit C and Lot no: 23325, Kit C), according to the validated test guideline for *in vitro* skin irritation, OECD TG 439 [54](OECD 2015). This model is comprised of non-transformed human-derived epidermal keratinocytes, which have been cultured to form a multi-layered, highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multi-layered stratum corneum containing intercellular lamellar lipid layers representing the main lipid classes analogous to those found *in vivo*.

Samples of Cosmetic B simulants from migration cells were applied topically to the EpiDerm™ with the use of a nylon mesh as a spreading aid for a 60 min exposure duration at 37°C. Pre-incubation and post-incubation washing steps were adhered to as optimized for the EpiDerm™ SIT (EPI-200) skin model. Samples of Cosmetic B simulant only were used as negative controls and a 5% sodium dodecyl sulfate (SDS) acted as a positive control (PC). Following exposure, inserts were thoroughly

washed according to the manufacturer's instructions (15 washes per insert followed by full immersion in Dulbecco's phosphate buffered saline (DPBS)) to remove all of the test material. The 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) assay was used to quantify viability based on the dehydrogenase activity of the mitochondria, and the conversion of MTT into blue formazan by the viable cells was measured at absorbance 570 nm according to test guidelines. Data are presented as percentage of viability measured in tissues exposed to Cosmetic B only. Cosmetic B simulants were chosen for testing as the highest levels of total overall migration was calculated in these simulants for PLA polymers and nanocomposite.

Supernatants of exposed tissues were analysed for levels of IL-8 and IL-1α as biomarkers of skin irritation using a bio-plex MAGPIX multiplex system (Luminex XmAP technology, Bio-Rad Laboratories) according to the recommended protocol from the manufacturer. Samples were measured in triplicate, and blank values were subtracted from all readings. Cytokine concentrations were interpolated from curves generated from reference standards and data are expressed as picograms per millilitre.

3. Results

3.1 Characterisation of PLA-organoclay nanocomposites

Wide angle X-ray diffraction (XRD) measurements were used to investigate the organoclay dispersion and exfoliation state (i.e. interlayer spacing of the clays and polymer intercalation) within the PLA polymer-organoclay nanocomposites. XRD diffractogram patterns comparing PLA2_HDTA, PLA2_TMSA organoclay nanocomposites and unmodified clay nanocomposite are presented in Figure 1. A single diffraction peak measured for the unmodified clay nanocomposite (PLA2_Unmodified) indicates a poor degree of clay dispersion in the PLA matrix, characteristic of phase separated large clay particles. In contrast, multiple diffraction peaks were measured for

nanocomposites with organoclays (PLA2_HDTA and PLA2_TMSA (Figure 1)) which indicates the presence of multiple clay particles with different crystalline phases intercalated within the polymer (PLA) matrix.

The interlayer d-spacing (the distance between the basal layers of the clay) was calculated from the XRD patterns and took into consideration Bragg's law (Table 1). A single diffraction peak at 7.68 2θ corresponds to a basal d-spacing of 11.5 Å for the N116 parent clays in the nanocomposites. This is similar to the interlayer spacing of N116 clays when in powder form and indicates a poor degree of polymer chain interaction between clay layers. The interlayer spacing calculated for the multiple diffraction peaks measured for the organoclay in composites ranged from 11.09-30.29 Å and 8.63-30.13 Å for HDTA and TMSA organoclays, respectively. The presence of larger interlayer spacing in organoclays within composites compared to organoclays in powder form indicate that the polymer has entered into the clay gallery within the composite, expanding the layers further with some degree of intercalation. The concentration of organic modifier used to organically modify the organoclays (1, 2, 4, 6, 8 times the excess of theoretical amount of modifier to exchange all the available cations from the clay) did not influence the dispersion state of the organoclays within the composite as a similar XRD diffraction pattern was observed (data not shown).

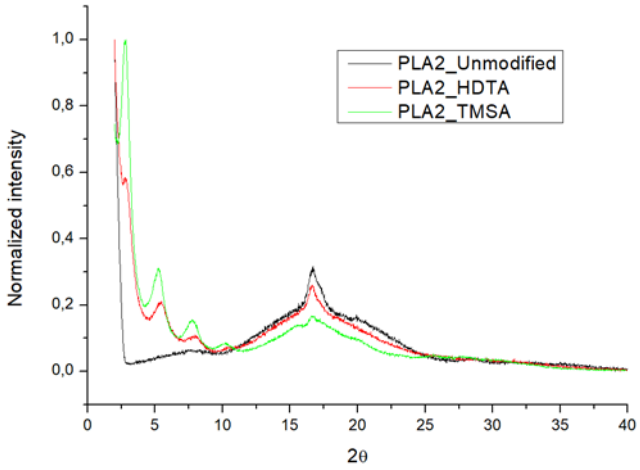

Figure 1. XRD pattern of PLA nanocomposites with unmodified clay (PLA2_Unmodified) and organoclays with HDTA and TMSA. 4 % (w/w) clay incorporation level was used in all cases.

Table 1. Characterisation of the structure of clays in powder form and within nanocomposites. Data expressed as average interlayer distance (Å), as measured using XRD (n=3).

Sample	Interlayer distance d_{001}(Å)	
	In powder form	Within PLA nanocomposite
N116	10.39	11.50
N116_HDTA	18.78	11.09/ 16.23/30.29
N116_TMSA	21.53	8.63/11.38/16.85/30.13

Light microscope images taken of polymers only and nanocomposites discs showed the continuous polymer matrix phase of extruded (PLA1) and injection moulded (PLA2) samples (Figures 2 (a) and (d), respectively) and the dispersion state of the respective organoclays after incorporation. A clear difference in dispersion state of the organoclays can be evidenced according to the processing technique used. Images of the PLA1 nanocomposites processed using extrusion are presented in Figures 2 (b) and (c). HDTA and TMSA organoclays can be seen evenly dispersed throughout the polymer matrix phase. In contrast a heterogenous distribution of different sized organoclays can be seen for PLA2 injection moulded nanocomposites (Figures 2 (e) and (f)). Large clay tactoids ≥45 µm in size can be viewed both for HDTA and TMSA organoclays. These tactoids are composed of individual nanoscale sheets agglomerated in stacks.

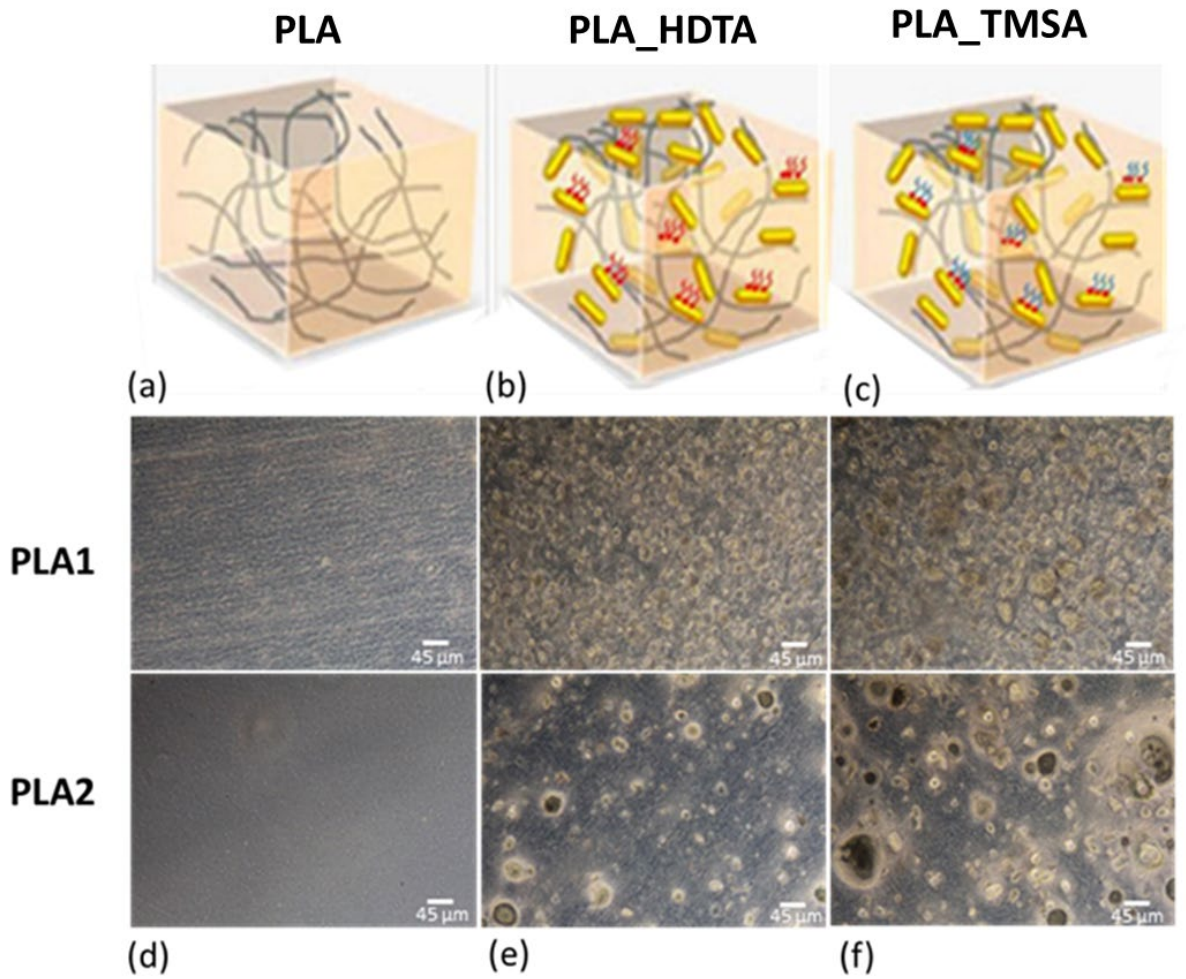

Figure 2. Diagrammatic representation and light microscopy images of the PLA polymers and PLA-clay nanocomposites incorporating HDTA and TMSA modified organoclays (4 % wt/wt respectively). Grey lines: polymer chain phase, Yellow blocks: reinforcing clay phase with HDTA (red tail and head group), or TMSA (blue tail and red head group) organic modification.

The mechanical properties of the PLA and PLA nanocomposites were investigated via assessment of the Young's Modulus. It was observed that the Young's Modulus was increased for PLA nanocomposites (PLA1_TMSA=2.924 GPa; PLA1_HDTA=3.118 GPa; PLA2_TMSA=3.542 GPa; PLA2_HDTA=3.707 GPa) compared to the pure PLA polymers (PLA1=2.691 GPa; PLA2=3.175 GPa). In the case of PLA2 nanocomposites, an increase in the elongation at break was also observed (PLA2_TMSA=2.041 mm; PLA2_HDTA=4.752 mm) with respect to the pure PLA2 polymer (1.677 mm). In the case of PLA1 nanocomposite, the elongation at break was also increased with respect to

the pure PLA1 polymer, but only when organoclays modified with TMSA were incorporated (PLA1=64.213 mm v´s PLA1_TMSA= 74.902 mm). In the PLA1 nanocomposites with organoclays modified with HDTA, the elongation at break was reduced by half to 31.838 mm.

3.2 In vitro biocompatibility studies

Using a direct contact method (IS0 10993-5) HaCaT skin cells were grown directly on PLA polymer and PLA polymer-organoclay nanocomposite discs and cell viability was assessed after 24 and 48 h using the alamarBlue® assay (Figure 3). There were no significant differences in the level of cell viability between cells grown on PLA polymers or nanocomposite discs when compared to control cells grown directly on the culture plate or on plastic coverslips (C) (Figure 3). Cell viability remained high (≥80 %) even after 48h incubation. Visual observation of cells using light microscopy and neutral red staining at 24h post exposure showed that both PLA polymers and nanocomposites supported cell attachment and monolayer growth with no visible indications of cytotoxicity after 24 h (Figure 4). Organoclays can be visualised on the surface of polymer nanocomposites.

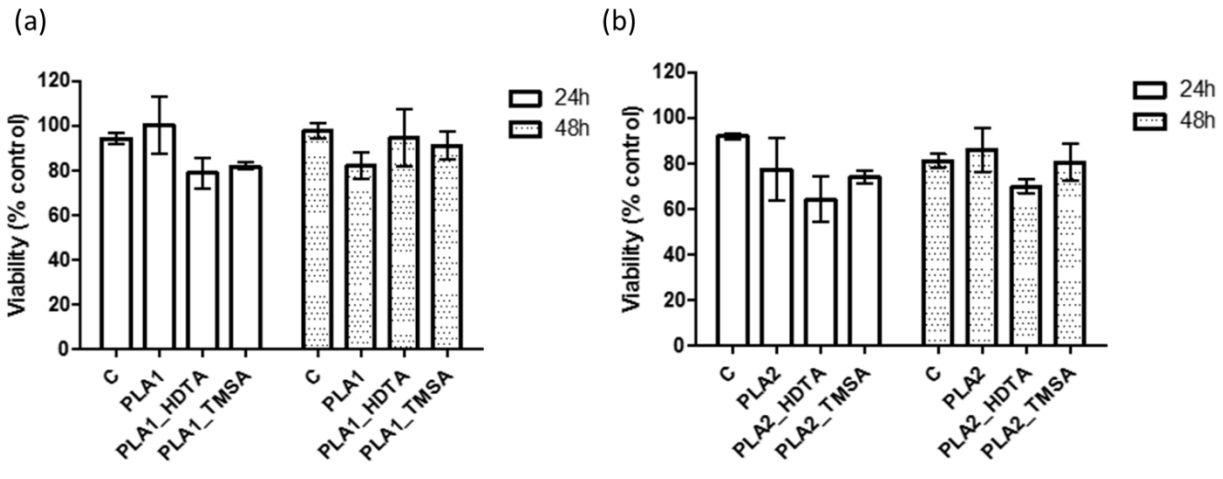

Figure 3. Viability of HaCaT skin cells in direct contact with PLA1 and nanocomposites (a) and PLA2 and nanocomposites (c) for a 24 and 48 h hour incubation period. C represents cells grown on plastic coverslips routinely used for cell culture. Data expressed as mean percentage viability levels compared to control cells grown directly on the culture plate ± SEM (n=3).

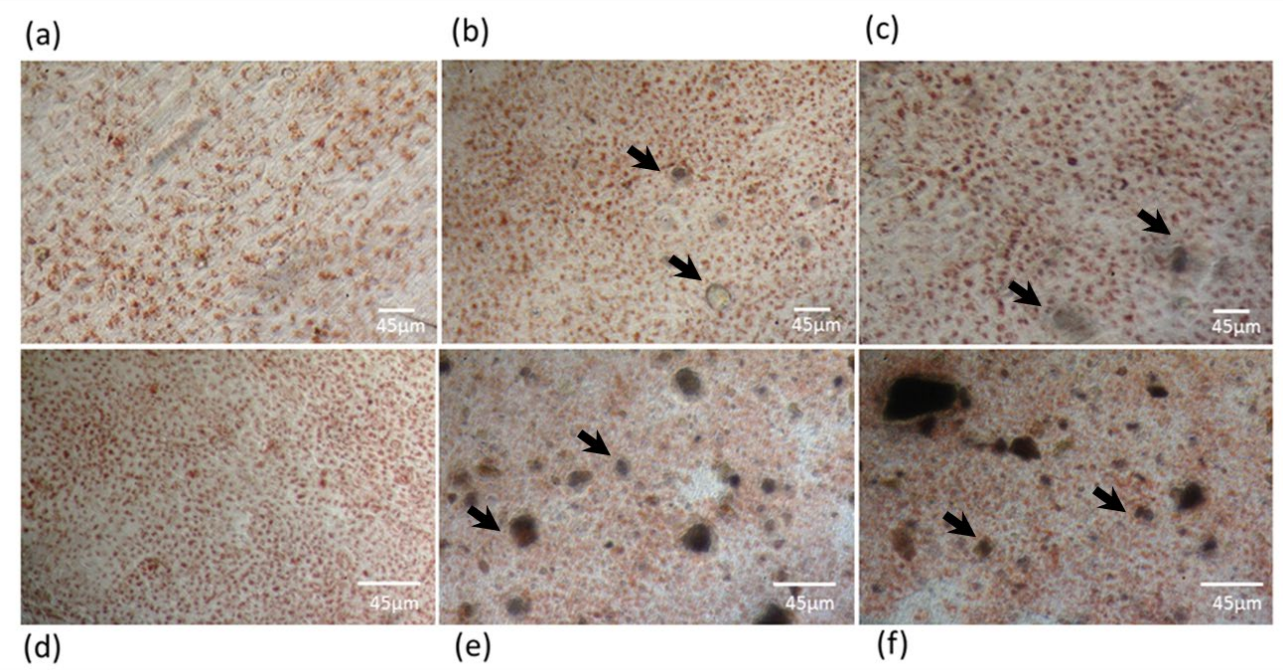

Figure 4. Light microscope images of HaCaT skin cells grown on PLA polymers (PLA1, PLA2) (a, and d respectively) and PLA nanocomposites; PLA1_HDTA (b), PLA2_HDTA (e), PLA1_TMSA (c), PLA2_TMSA (f) (24h post incubation). Black arrows point to some clearly visible organoclays seen on surface of nanocomposites.

3.3 Migration

Total overall migration levels were calculated for PLA polymer and PLA polymer-organoclay nanocomposite discs according to measured differences in weights prior to and after being in contact with a range of simulants for 10 days at 40°C. Following 10 days in contact with the

cosmetics at 40°C, all polymer and polymer organoclay nanocomposite samples remained intact, with no visible signs of degradation. There was also no obvious change in the appearance of the simulants. In Figure 5a the migration levels for PLA1 and nanocomposites in two different cosmetic formulations, vegetable oil (simulant D) and MEM aqueous based elution culture medium are presented and compared.

Migration from the PLA1 polymer ranged from 0.27±0.14 to 0.66±0.33 mg/dm^2 according to the simulant used (Figure 5). Migration from PLA1 polymer-organoclay nanocomposites (PLA1_HDTA and PLA1_TMSA) was 0.47±0.34 to 0.88±0.44 mg/dm^2 respectively. No appreciable levels of migratoin were measured in simulant D for any of the test substances. The highest levels of migration was measured for the PLA1_TMSA nanocomposite in Cosmetic B and MEM elution medium (0.86±0.43 and 0.88±0.44 mg/dm^2 , respectively).

In contrast, there was no appreciable migration from PLA2 polymer samples measured in Cosmetic B or simulant D (Figure 5b) and low migration levels in Cosmetic A and MEM elution medium (0.17±0.09 mg/dm^2 and 0.32±0.16 mg/dm^2, respectively). Similar levels of migration were measured for PLA2 and PLA2_HDTA nanocomposites in MEM elution medium, but again the highest level of migration was measured for PLA1_TMSA nanocomposites (0.85 ±0.43 mg/dm^2).

It is important to note that all migration levels are well below the overall migration limit of 10 mg/dm^2 set by the EU-Plastics Regulation 10/2011 [51](EU 2011). Negative migration levels were also calculated for samples which increased in weight following contact with simulants (Figure 5).

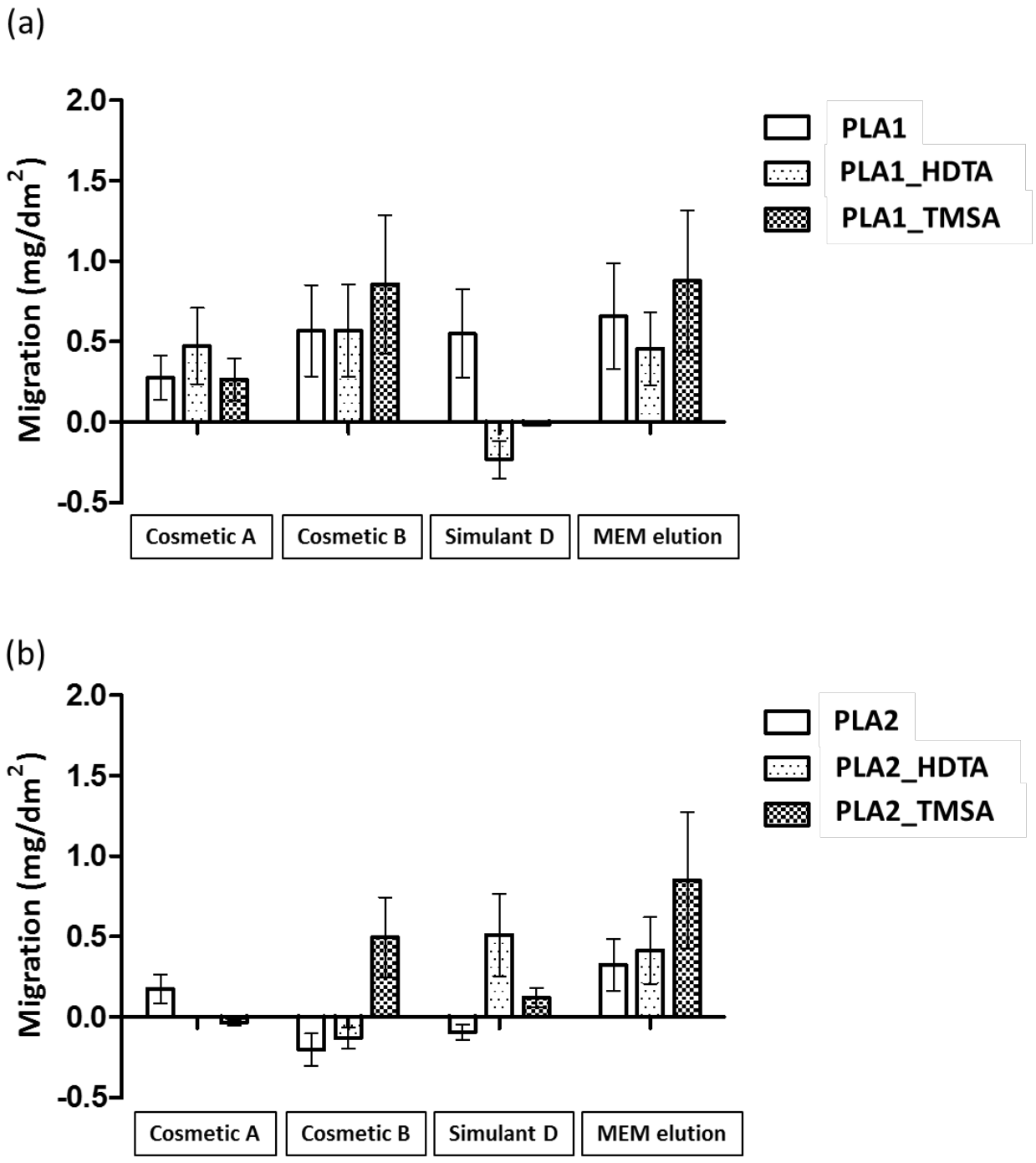

Figure 5. Total overall migration levels measured for PLA1 polymers and nanocomposites (a) and PLA2 polymers and nanocomposites (b) in a range of simulants following contact for 10 days and incubation at 40 °C. Data are expressed as mean ± SEM (n= 4). The absence of bars for specific samples indicate that there was no change in sample weight prior to and after being in contact with simulant. Negative values were calculated for samples that increased in weight in migration tests.

3.4 *In vitro* hazard assessment of migration extracts

The cytotoxicity of MEM simulant migration extracts from the PLA polymers and PLA polymer-organoclay nanocomposites towards HaCaT skin cells was tested *in vitro*. As a control, cells were exposed to the MEM elution simulant that had been in contact with sterile plastic coverslips. Cell viability 24 h post exposure to MEM migration extracts from PLA1 polymers and PLA1 polymer-organoclay nanocomposites ranged from 83±16% to 96±13%, compared to control cells grown in the MEM elution blank (simulant only). (Figure 6). Viability of cells grown in contact with PLA2 and PLA2 polymer-organoclay nanocomposites migration extracts were between 98±8%-113±13% of control levels. There were no significant differences in cell viability for the migration extracts of any of the test substances (i.e. PLA1, PLA1_HDTA, PLA1_TMSA, PLA2, PLA2_HDTA, PLA2_TMSA), compared to the control.

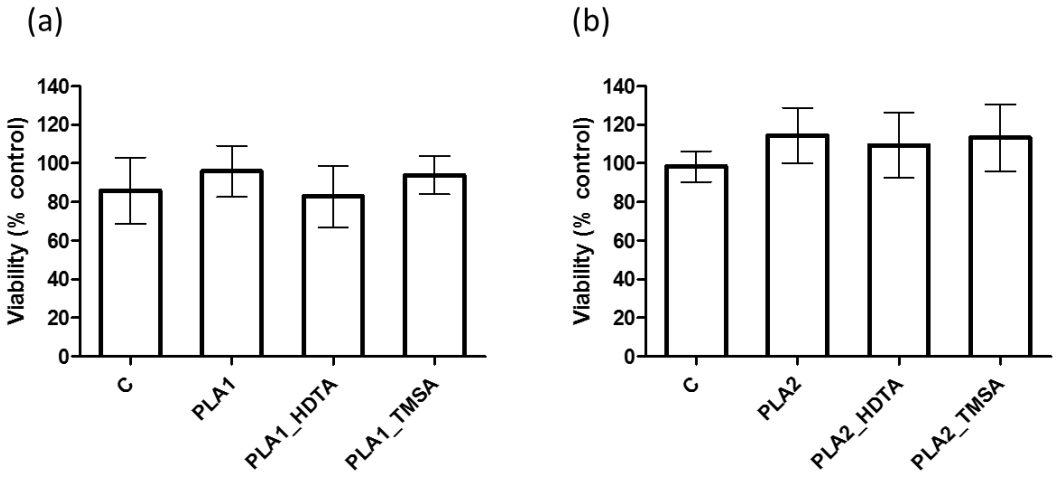

Figure 6. Cytotoxicity assessment of migration extracts in MEM elution simulants in contact with PLA1 and PLA1 polymer-organoclay nanocomposites (a) and PLA2 and PLA2 polymer-organoclay nanocomposites (b) using HaCaT skin cells. Cell viability was assessed using the alamarBlue® assay. Data are expressed as mean percentage viability compared to control cells exposed to MEM elution medium ± SD (n= 4). C represents cells exposed to MEM elution simulants in contact with sterile plastic coverslips.

Assessment of the toxicity of the migration extracts of the PLA polymers, and PLA polymer-organoclay nanocomposites in Cosmetic B was prioritised as a higher level of migration was observed in the simulant compared to Cosmetic A. The migration extract from the PLA polymers, and PLA polymer-organoclay nanocomposites in Cosmetic B was tested for skin irritation properties using an *in vitro* reconstructed human skin epidermal model, EpiDerm^{TM}. The cosmetic formulation was applied topically to the models for 60 min and viability of the epidermal keratinocytes within the model was assessed using the MTT assay, according to test guidelines. There was no significant reduction in cell viability in skin tissue models exposed to Cosmetic B migration extracts from PLA1 or PLA2 polymers or nanocomposites (Figure 7a). Levels of viability remained between 78±6 to 92±4 % of measured levels for untreated control cells. Migration extracts can be considered non-irritating according to the threshold value of ≤ 50%, set for UN GHS Category 2 classification as a skin irritant. Viability of tissues exposed to SDS (5%) which acted as a positive control were reduced to levels ≤ 20%.

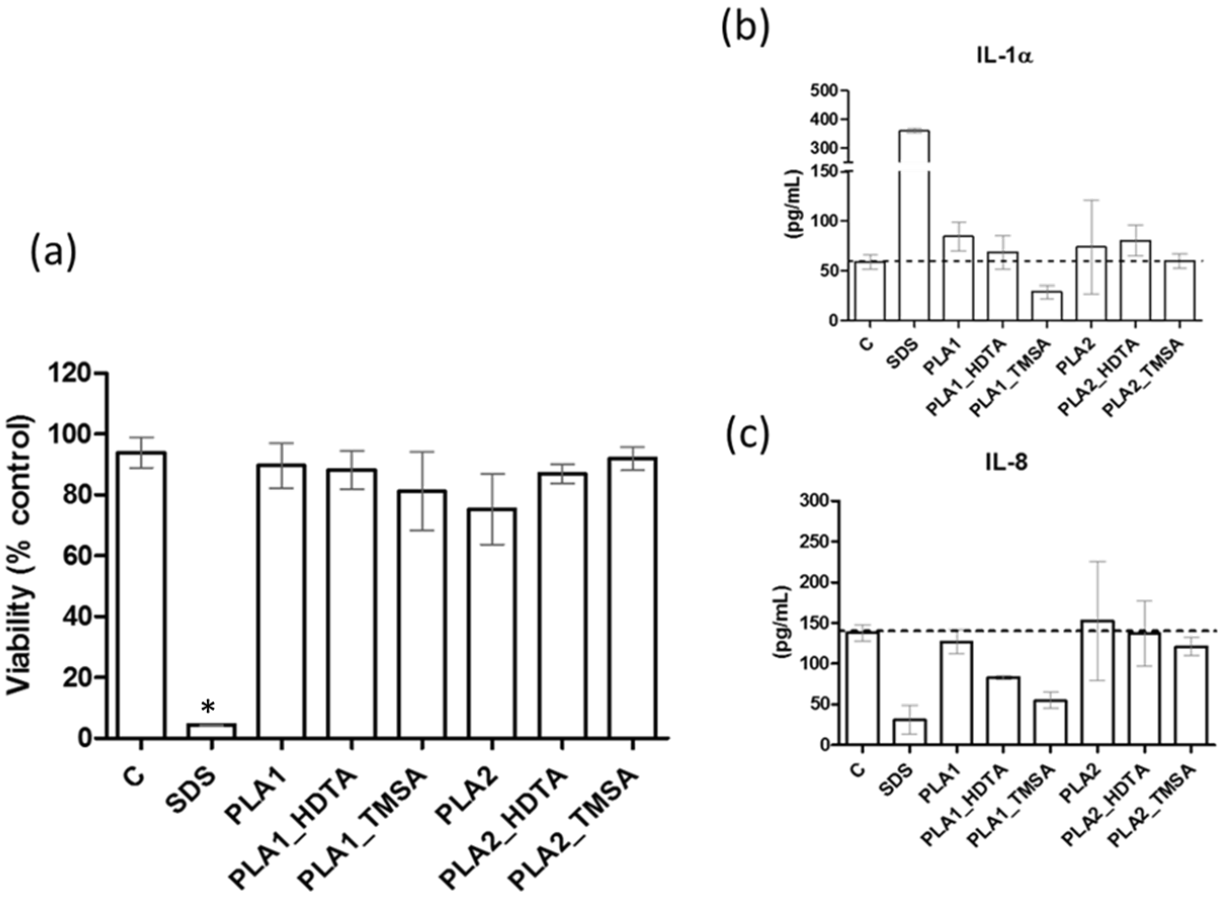

Figure 7. Analysis of skin irritation potential of migration extracts in cosmetic B (simulant) using the EpiDerm™ skin model and assessing viability (a) and cytokine release; IL-α (b) and IL-8 (c). Data are expressed in mean ± SD (n= 3). PC; positive control (5% SDS), C; cosmetic B exposure.

The analysis of pro-inflammatory cytokines, IL-1α and IL-8, as biomarkers of potential irritation or sensitising reactions within a hazard assessment has been proposed [55](Coquette et al. 2003) and in this study was used in conjunction with OECD TG 439. Levels of IL-1α were only significantly increased, compared to control cells, in tissues exposed to the positive control (5% SDS), but no change in cytokine production was observed for the other test substances (Figure 7b). No change in IL-8 production was observed for any treatment, compared to the control (Figure 7c).

4. Discussion

The use of bio-based and biodegradable PLA polymer nanocomposites as alternatives to petroleum-based plastics has the potential to revolutionise the packaging industry. There are concerns however surrounding safety, and the potential for components (e.g. clays) to migrate from such packaging must be addressed. This study was performed to assess the safe use of novel PLA polymer-organoclay nanocomposites as packaging materials in the cosmetic industry via an investigation into the potential migration of constituents (clays, organic modifiers, lactide, lactic acid and oligomers) and the potential hazard following dermal exposure. It is distinct from most reported studies to date which have focused on polymer nanocomposites use as food packaging and that have assessed risks from the oral route of exposure. Up until now, the hazard of migration extracts from polymer-organoclay nanocomposites following dermal exposure has not been investigated and thus our study addresses a gap in knowledge. The polymer-organoclay nanocomposites under investigation in this study have been produced from commercially available PLA polymers reinforced with organically modified bentonite clays (which were incorporated to improve gas barrier properties and strength). In total four nanocomposites were produced (PLA1_HDTA, PLA1_TMSA, PLA2_HDTA, and PLA2_TMSA) and tested for their biocompatibility, migration potential and the ability of migration extracts to elicit adverse effects to HaCaT skin cells (*in vitro*) and irritation potential using 3D *in vitro* skin models. Different grades of polymers (PLA1 and PLA2), different processing technique (extrusion and injection moulding) and two different organoclay nanofillers (HDTA and TMSA modified) were used.

4.1 Nanocomposite structure: influence of properties on safety and migration potential

Many factors are likely to have a direct influence on polymer nanocomposite stability and the migration potential of components including polymer type, the processing mechanism used, the thickness of the final nanocomposite, the concentration and dispersion state of the clay incorporated into the polymer matrix, as well as the type of simulant in contact with the material.

The PLA polymers used in this study have been commercialised (Ingeo™ Biopolymer 3052D, 2003D) and specifically designed for use within the food industry for applications in food packaging and

plastic tableware. Two different semi-crystalline polymer grades were selected according to their processing capabilities for injection moulding and extrusion, respectively. The PLA grades had the same density (1.25 g/cm^{-3} vs and 1.24 g/cm^{-3}) and in general similar characteristics according to manufacturer's datasheets. However, there is a difference in thickness of the finished PLA nanocomposite samples produced in this study due to the specimen format for each PLA grade obtained by different techniques (extruded sheets and injected discs) (0.4 and 0.8 mm, for PLA1 and PLA2 nanocomposites, respectively). The extent to which this property influenced migration of nanocomposites is unclear. However, the obtained data demonstrates that a greater level of migration was observed in the thinner PLA1 nanocomposites, in all simulants tested. In contrast, other authors have reported equivalent levels of migration in ethanol for polypropylene (PP)-clay films of different thickness (22.5 vs 45.6 μm) [40](Xia et al. 2014).

The effect of clay incorporation levels on migration has been investigated and often with contradictory results. Some studies show an increase in total overall migration with increasing loading (1.8-5%) [56](Schmidt et al. 2011), however others report no direct relationship between increased clay content and increased release [40](Xia et al. 2014) and instead suggest that the degree of interaction between the clays and polymer influence the mobility of the clay in the nanocomposite. The same level of organoclay incorporation (4 % wt/wt) was used for all PLA nanocomposites under investigation in this study. This represents a relatively low incorporation level; with up to 10 % being reported previously [14](Chang et al. 2003). XRD analysis revealed increases in interlayer spacing (30.29 Å and 30.13 Å for PLA_HDTA and PLA_TMSA respectively), which is an indication of polymer interaction for both clays within composites and therefore the likely presence of smaller clay particle sizes. Clays were organically modified to improve interaction and thus this is expected. Whilst interfacial interactions exist between the polymer chains and the clay in nanocomposites, they are not covalently bound to the plastic matrix and therefore should not be thought of as fixed, but instead partitioned. Clay size and the dynamic viscosity of the polymer matrix are therefore significant contributing factors in migration potential as discussed by [57] Simon

et al. (2008) with the highest potential migration proposed for small nanomaterials (<1nm) from polymer matrices with low dynamic viscosities. In this study clays with particle sizes ≤45 μm (in powder form) were incorporated at the polymer processing step however following processing the extent of interaction of clays with the polymer matrix will directly influence particle size and can lead to exfoliated clay platelets present within the matrix.

The different processing techniques used had a direct impact on the dispersion of the organoclay filler within the finished nanocomposite. The use of a plastisizer in PLA1 polymer nanocomposite processing may have influenced the dispersion of clays within the polymer matrix. For example, it was demonstrated previously that increasing concentrations of the plasticiser triethyl citrate (TEC) improved the distribution of chitin nanocrystals in PLA nanocomposites [58](Herrera et al. 2017). Any loss in organic modifier from clay interlayers during processing (e.g. thermal degradation of organic modifiers at high temperature) has been shown to impede intercalation and influence the dispersion and distribution state [59, 60](Shah et al. 2006, Xie et al. 2001) and may explain the phase separated distribution in PLA2 samples. If this is the case free organic modifier or its degradation product(s) are likely to be present within the composite. While organoclays with relatively low surfactant loadings (23-26% w/w) were used in this study, loadings of surfactant in commercially available modified clays can be as high as 34-36 % wt (Nanomer1.44P, from Minerals Technologies Inc.). It is thus important also to assess the potential for organic modifier to migrate from polymer nanocomposites in a safety assessment.

Another important consideration is the potential release of polymer nanocomposite components (e.g. clays) as a result of the degradation of the polymer matrix, which has been discussed in detail in a recent review [61](Duncan 2015). Potential degradation by hydrolysis of the PLA nanocomposites being investigated in this study is particularly relevant, and may lead to toxicity as the solvents and other ingredients commonly found in cosmetic formulations may enhance PLA hydrolysis. Two cosmetic formulations were used within this study as simulants to represent a simple formulation

(Cosmetic A) and a highly lipophilic emulsion (Cosmetic B). The greatest total overall migration was seen for polymer nanocomposites in contact with Cosmetic B for both injection-moulded and extruded samples. Interestingly a loss in weight was also measured for PLA polymer samples (i.e. in the absence of organoclays), suggesting migration of components such as lactic acid passively or as a result of polymer degradation in contact with Cosmetic B. The ease of decomposition of PLA in other simulants such as water, 3% acetic acid and 20% ethanol to lactide, lactic acid and oligomers has been evidenced with higher decomposition rates at higher temperatures (>60^{o}C) [62](Mutsuga et al. 2008). Similar migration profiles for PLA1 polymers and nanocomposites in Cosmetic B and vegetable oil simulants suggest the lipophilic nature of the simulants are controlling migration. In contrast, lower migration levels were measured from PLA1 nanocomposites in less lipophilic simulants (e.g. Cosmetic A). While elucidating the exact mechanism of release/migration is out of the scope of this study a recent study argues that the affinity between the simulant and polymer is the dominant factor controlling migration [63](Nasiri et al. 2016).

4.2 Migration

Components of the polymer and polymer organoclay nanocomposites can migrate from the packaging, including for example lactic acid/lactide monomers, organoclays and or HDTA/TMSA organic modifiers. Accordingly, PLA and nanocomposite samples were placed in contact with a range of simulants including; cosmetic formulations to mimic real use scenarios, vegetable oil to represent a fatty simulant and aqueous based MEM as an elution test medium, and the levels of migration assessed according to a decrease in polymer weight. Total overall migration (TOM) levels were calculated by taking into consideration any loss in weight of the polymer and polymer organoclay nanocomposite sample as a function of their surface area in dm^2. TOM levels of 0.17±0.09 to 0.66±0.33 mg/dm^2 were calculated from PLA polymers only. The migration of lactide, lactic acid and low molecular weight oligomers from PLA polymer itself at the same level (1 mg/dm^2) has also been

reported previously [62](Mutsuga et al. 2008). The highest TOM levels were calculated for PLA1_TMSA with a measured reduction in weight equivalent to a migration level into the cosmetic of 0.86± 0.43 mg/dm^2. This represented only a slight increase from levels of migration form PLA1 only (0.57 ±0.28 mg/dm^2).

Overall migration values of 0.1 ± 0.2 mg/dm^2 have been reported for PLA nanocomposite bottles with 4 % w/w HDTA modified nanoclays held under similar conditions (40^{o}C, 10 days) using distilled water as a simulant [64](Maisanaba et al. 2015). Higher migration levels from PLA nanocomposites (PLA/5% Cloisite®30B) compared to PLA only polymers in 95% ethanol simulants have been reported (6.7 mg/dm^2 v's 1.7 mg/dm^2) [65](Schmidt et al. 2009). It is important to point out that taken together all migration levels previously reported and calculated in this study are well below the overall migration limit of 10 mg/dm^2 set by the EU- Plastics Regulation 10/2011 [51](EC 2011).

There are challenges in identifying the migration of materials from polymer and polymer organoclay nanocomposites due to limitations in analytical techniques that can identify individual clay particles, especially in complex matrices such as a cosmetic formulation. While the analysis of migration extracts is outside the scope of this study the release of both surfactants and nanomaterials from nanocomposites has been reported previously [40](Xia et al. 2014) using liquid chromatography tandem mass spectrometry and graphite furnace atomic absorption spectrometry respectively. Furthermore, fluorescent labelling has been explored using fluorescein and rhodamine labelling to track migration of nanoclays from polymers into solvents (eg. ethanol) [66](Diaz et al. 2013).

4.3 Nanocomposite safety: Investigating possible adverse effects from migration extracts

Prior to this investigation the dermal toxicity of migration extracts from PLA-organoclay nanocomposites was unknown. Early investigations have reported on the safe use of PLA for food packaging according to the low risk to consumer health following ingestion of PLA migration products (lactic acid, and its dimers and oligomers) [67](Conn et al. 1995).

In this study migration from the PLA polymers was evident, particularly for PLA1 extruded polymers. However, these migration products are likely to represent a low risk to the consumer following dermal exposure due to a lack of evidence of cytotoxicity observed in HaCaT keratinocytes or irritation in 3D *in vitro* skin models. Indeed, lactic acid is a commonly used cosmetic ingredient approved for use in skin conditioning agents and exfoliants at concentrations from 2.5 to 10 % (EC, SCCNFP[2], FDA).

PLA polymers are approved for use in biomedical applications [68](Tyler et al. 2016) however when clays are added to such polymers, they can have adverse effects on the sample's biocompatibility [69](Zia et al. 2011). In addition, while unmodified bentonite clays are approved for use as colorants in cosmetic products, organic modification has been shown to increase the hazard potential of these materials [70,38,39](Maisanaba et al. 2014, Wagner et al. 2017, Connolly et al. 2019). It has been suggested that organoclays should only be used in industries where surfactant migration is acceptable and non-toxic, due to their biocidal nature [71](Nigmatullin et al 2008). Therefore, it was important to test if migration extracts were hazardous in the application of such PLA polymer-organoclay nanocomposites in the cosmetic packaging industry. The *in vitro* biocompatibility of the PLA nanocomposites was tested via assessment of HaCaT skin cell growth on polymer and polymer organoclay nanocomposites. All test samples supported cell attachment and growth and no effects on levels of viability were seen. While calculated TOM levels show migration of components in an aqueous MEM medium simulant for both PLA1 and PLA2 polymers and nanocomposites, there was no evidence of a reduction in HaCaT skin cell viability when exposed to migration extracts. Furthermore, to mimic a real exposure scenario, migration extracts in the cosmetic formulations used as simulants during migration studies (Cosmetic B) were applied topically to the reconstructed EpiDerm^{TM} skin model. Results revealed no indication of skin irritation potential assessed according to OECD TG 439 and monitoring of levels of pro-inflammatory cytokines (IL-1α and IL-8).

[2] Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP):

Taken together the results therefore suggest that the concentration at which migration extracts were present in the cosmetic simulant did not cause any adverse effects.

5. Conclusion

This study was performed to assess the suitability/safety of PLA polymer-organoclay nanocomposites for use as packaging materials in the cosmetic industry. Most assessments performed to date have specifically focused on nanocomposites for use in the food packaging industry. However, there is also a large market potential for use of bio-packaging in the cosmetic industry. Results from this study indicate that there is likely to be minimal dermal toxicity associated with the use of the PLA-organoclay nanocomposites prepared in this study for cosmetic packaging according to the levels of migration measured (exposure) and dermal toxicity (hazard) assessments performed. Despite a lack of specific guidelines for migration of components from cosmetic packaging, existing guidelines for migration assessment for articles in contact with food packaging were followed. Migration of components of the polymer-organoclay nanocomposites was assessed in a range of simulants, with the incorporation of cosmetic formulations included to mimic real exposure conditions. It was observed that migration was controlled by both the polymer grade, processing type and simulant used, with higher migration in more lipophilic simulants. However, overall migration from the PLA nanocomposites was low (ranged from 0.26± 0.13 mg/dm^2 to 0.88± 0.44 mg/dm^2) and within permitted levels (<10 mg/dm^2). Biocompatibility of the PLA-organoclay nanocomposites, and toxicity of the migration components was assessed *in vitro*. No toxicity was observed for any of the PLA-organoclay nanocomposites tested. The data obtained suggests that the PLA nanocomposites developed can be used in cosmetic packaging, and the information can guide the safe design and development of bio-packaging for cosmetic (and other) industries in the future.

Competing interests and funding statements

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Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to legal or ethical reasons.

References

[1] D.R. Paul, L.M. Robeson, Polymer nanotechnology: Nanocomposites, Polymer (Guildf). 49 (2008) 3187–3204. doi:10.1016/j.polymer.2008.04.017.

[2] Plastics Europe, Plastics - the Facts 2017 An analysis of European plastics production, demand and waste data. 2016, Plastics Europe: Brussels. https://www.plasticseurope.org/application/files/5715/1717/4180/Plastics_the_facts_2017_FINAL_for_website_one_page.pdf (accessed 18 January 2019).

[3] M.M. Reddy, S. Vivekanandhan, M. Misra, S.K. Bhatia, A.K. Mohanty, Biobased plastics and bionanocomposites: Current status and future opportunities, Prog. Polym. Sci. 38 (2013) 1653–1689. doi:10.1016/j.progpolymsci.2013.05.006.

[4] S. Ray, International Journal of Food The Potential Use of Polymer-Clay Nanocomposites in Food Packaging The Potential Use of Polymer-Clay Nanocomposites in Food Packaging, Int. J. Food Eng. 2 (2006) 1–11. doi:10.2202/1556-3758.1149.

[5] R.P. John, K.M. Nampoothiri, A. Pandey, Fermentative production of lactic acid from biomass: An overview on process developments and future perspectives, Appl. Microbiol. Biotechnol. 74 (2007) 524–534. doi:10.1007/s00253-006-0779-6.

[6] V.M. Ghorpade, A. Gennadios, M.A. Hanna, Laboratory composting of extruded poly(lactic acid) sheets, Bioresour. Technol. 76 (2001) 57–61. doi:10.1016/S0960-8524(00)00077-8.

[7] Reports Database 2018-2023. Polylactic Acid (PLA) Market Global Key Player, Demand, Growth, Opportunities and Analysis Forecast. Wise guy reports database. https://www.marketwatch.com/press-release/global-polylactic-acid-pla-market-2018-industry-trend-and-forecast-2023-2018-05-31 (accessed 03 January 2019).

[8] European Bioplastics Report. Bioplastics market data 2017. Global production capacities of bioplastics 2017-2022. Available at https://docs.european-bioplastics.org/publications/market_data/2017/Report_Bioplastics_Market_Data_2017.pdf

[9] S. Sinha Ray, P. Maiti, M. Okamoto, K. Yamada, K. Ueda, New polylactide/layered silicate nanocomposites. 1. Preparation, characterization, and properties, Macromolecules. 35 (2002) 3104–3110. doi:10.1021/ma011613e.

[10] K. Prakalathan, S. Mohanty, S.K. Nayak, Polylactide/modified layered silicates nanocomposites: A critical analysis of morphological, mechanical and thermal properties, J. Reinf. Plast. Compos. 31 (2012) 1300–1310. doi:10.1177/0731684412457597.

[11] R.N. Darie, E. Pâslaru, A. Sdrobis, G.M. Pricope, G.E. Hitruc, A. Poiată, A. Baklavaridis, C. Vasile, Effect of nanoclay hydrophilicity on the poly(lactic acid)/clay nanocomposites properties, Ind. Eng. Chem. Res. 53 (2014) 7877–7890. doi:10.1021/ie500577m.

[12] M. Murariu, P. Dubois, PLA composites: From production to properties, Adv. Drug Deliv. Rev. 107 (2016) 17–46. doi:10.1016/j.addr.2016.04.003.

[13] M. Jorda-Beneyto, N. Ortuño, A. Devis, S. Aucejo, M. Puerto, D. Gutiérrez-Praena, J. Houtman, S. Pichardo, S. Maisanaba, A. Jos, Use of nanoclay platelets in food packaging materials: technical and cytotoxicity approach, Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 31 (2014) 354–363. doi:10.1080/19440049.2013.874045.

[14] J.H. Chang, Y.U. An, G.S. Sur, Poly(lactic acid) nanocomposites with various organoclays. I. Thermomechanical properties, morphology, and gas permeability, J. Polym. Sci. Part B Polym. Phys. 41 (2002) 94–103. doi:10.1002/polb.10349.

[15] J.W. Rhim, S.I. Hong, C.S. Ha, Tensile, water vapor barrier and antimicrobial properties of PLA/nanoclay composite films, LWT - Food Sci. Technol. 42 (2009) 612–617. doi:10.1016/j.lwt.2008.02.015.

[16] B. Şengül, N. Dilsiz, Barrier properties of polylactic acid/layered silicate nanocomposites for food contact applications, Polym. Sci. Ser. A. 56 (2014) 896–906. doi:10.1134/s0965545x14060194.

[17] P. Maiti, K. Yamada, M. Okamoto, K. Ueda, K. Okamoto, New polylactide/layered silicate nanocomposites: Role of organoclays, Chem. Mater. 14 (2002) 4654–4661. doi:10.1021/cm020391b.

[18] S. Sinha Ray, K. Yamada, M. Okamoto, K. Ueda, Biodegradable Polylactide/Montmorillonite Nanocomposites, J. Nanosci. Nanotechnol. 3 (2004) 503–510. doi:10.1166/jnn.2003.220.

[19] M. Alexandre, P. Dubois, Polymer layered silicate nanocomposites preparation properties, Mater. Sci. Eng. 28 (2000) 1–63. doi:10.1002/1439-2054(20000601)279:1<1::AID-MAME1>3.0.CO;2-Q.

[20] Dennis H.R., Hunter D.L., Chang D., Kim S., White J.L., Cho J.W., Paul Donald R., Effect of melt processing conditions on the extent of exfoliation in organoclay-based nanocomposites, Polymer (Guildf). 42 (2001) 9513–9522. doi:10.1016/S0032-3861(01)00473-6.

[21] P.C. Lebaron, Z. Wang, T.J. Pinnavaia, Polymer-layered silicate nanocomposites: An overview, Appl. Clay Sci. 15 (1999) 11–29. doi:10.1016/S0169-1317(99)00017-4.

[22] V. Siracusa, P. Rocculi, S. Romani, M.D. Rosa, Biodegradable polymers for food packaging: a review, Trends Food Sci. Technol. 19 (2008) 634–643. doi:10.1016/j.tifs.2008.07.003.

[23] D.M. Marquis, É. Guillaume, C. Chivas-Joly, Properties of Nanofillers in Polymer, in: Cuppoletti, J (Ed.), Nanocomposites and Polymers with Analytical Methods, InTech, Europe and China, 2011, pp. 281–304.

[24] M.A. Youssef, Polymer Nanocomposites as a New Trend for Packaging Applications, Polym. Plast. Technol. Eng. 52 (2013) 635–660. doi.org/10.1080/03602559.2012.762673.

[25] J.W. Rhim, H.M. Park, C.S. Ha, Bio-nanocomposites for food packaging applications, Prog. Polym. Sci. 42 (2013) 1629–1652. doi.org/10.1016/j.progpolymsci.2013.05.008.

[26] H.M.C. d. Azeredo, Nanocomposites for food packaging applications, Food Res. Int. 42 (2009) 1240–1253. doi:10.1016/j.foodres.2009.03.019.

[27] J. Bošnir, D. Puntarić, A. Galić, I. Škes, T. Dijanić, M. Klarić, M. Grgić, M. Čurković, Z. Šmit, Migration of phthalates from plastic containers into soft drinks and mineral water, Food Technol. Biotechnol. 45 (2007) 91–95.

[28] C. Bach, X. Dauchy, M.C. Chagnon, S. Etienne, Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: A source of controversy reviewed, Water Res. 46 (2012) 571–583. doi:10.1016/j.watres.2011.11.062.

[29] E. Fasano, F. Bono-Blay, T. Cirillo, P. Montuori, S. Lacorte, Migration of phthalates, alkylphenols, bisphenol A and di(2-ethylhexyl)adipate from food packaging, Food Control. 27 (2012) 132–138. doi:10.1016/j.foodcont.2012.03.005.

[30] D. Zalko, C. Jacques, H. Duplan, S. Bruel, E. Perdu, Viable skin efficiently absorbs and metabolizes bisphenol A, Chemosphere. 82 (2011) 424–430. doi:10.1016/j.chemosphere.2010.09.058.

[31] European Union Commission Regulation 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materials.

[32] F. Uddin, Montmorillonite: An Introduction to Properties and Utilisation, in: M. Zoveidavianpoor (Ed.) Current Topics in the Utilization of Clay in Industrial and Medical Applications, Intech, Europe and China, 2018 pp. 3–23.

[33] S. Xu, Z. Zhang, M. Chu, Long-term toxicity of reduced graphene oxide nanosheets: Effects on female mouse reproductive ability and offspring development, Biomaterials. 54 (2015) 188–200. doi:10.1016/j.biomaterials.2015.03.015.

[34] K. Donaldson, F.Murphy, A. Schinwald, R. Duffin, C.A. Poland, Identifying the pulmonary hazard of high aspect ratio nanoparticles to enable their safety-by-design, Nanomedicine. 6 (2010) 143–156. doi.org/10.2217/nnm.10.139.

[35] T. Xia, Y. Zhu, L. Mu, Z.F. Zhang, S. Liu, Pulmonary diseases induced by ambient ultrafine and engineered nanoparticles in twenty-first century, Natl. Sci. Rev. 3 (2017) 416–429. doi:10.1093/nsr/nww064.

[36] G. Janer, E. Fernández-Rosas, E. Mas del Molino, D. González-Gálvez, G. Vilar, C. López-Iglesias, V. Ermini, S. Vázquez-Campos, In vitro toxicity of functionalised nanoclays is mainly driven by the presence of organic modifiers, Nanotoxicology. 8 (2014) 279–294. doi:10.3109/17435390.2013.776123.

[37] S. Lordan, J.E. Kennedy, C.L. Higginbotham, Cytotoxic effects induced by unmodified and organically modified nanoclays in the human hepatic HepG2 cell line, J. Appl. Toxicol. 31 (2011) 27–35. doi:10.1002/jat.1564.

[38] A. Wagner, R. Eldawud, A. White, S. Agarwal, T.A. Stueckle, K.A. Sierros, Y. Rojanasakul, R.K. Gupta, C.Z. Dinu, Toxicity evaluations of nanoclays and thermally degraded byproducts through spectroscopical and microscopical approaches, Biochim. Biophys. Acta - Gen. Subj. 1861 (2017) 3406–3415. doi:10.1016/j.bbagen.2016.09.003.

[39] M. Connolly, Y. Zhang, S. Mahri, D.M. Brown, N. Ortuño, M. Jordá-Beneyto, K. Maciaszek, V. Stone, T.F. Fernandes, H.J. Johnston, The influence of organic modification on the cytotoxicity of clay particles to keratinocytes, hepatocytes and macrophages; an investigation towards the safe use of polymer-clay nanocomposite packaging, Food Chem. Toxicol. 126 (2019) 178–191. doi:10.1016/j.fct.2019.02.015.

[40] Y. Xia, M. Rubino, R. Auras, Release of nanoclay and surfactant from polymer-clay nanocomposites into a food simulant, Environ. Sci. Technol. 48 (2014) 13617–13624. doi:10.1021/es502622c.

[41] C.P. Gerba, Quaternary ammonium biocides: Efficacy in application, Appl. Environ. Microbiol. 81 (2015) 464–469. doi:10.1128/AEM.02633-14.

[42] G.H.Y. Lin, M. Hemming, Ocular and dermal irritation studies of some quaternary ammonium compounds, Food Chem. Toxicol. 34 (1996) 177–182. doi:10.1016/0278-6915(95)00099-2.

[43] H. Nagamune, T. Maeda, K. Ohkura, K. Yamamoto, M. Nakajima, H. Kourai, Evaluation of the cytotoxic effects of bis-quaternary ammonium antimicrobial reagents on human cells, Toxicol. Vitr. 14 (2000) 139–147. doi:10.1016/S0887-2333(00)00003-5.

[44] F. Ferk, M. Mišík, C. Hoelzl, M. Uhl, M. Fuerhacker, B. Grillitsch, W. Parzefall, A. Nersesyan, K. Mičieta, T. Grummt, V. Ehrlich, S. Knasmüller, Benzalkonium chloride (BAC) and dimethyldioctadecyl-ammonium bromide (DDAB), two common quaternary ammonium compounds, cause genotoxic effects in mammalian and plant cells at environmentally relevant concentrations, Mutagenesis. 22 (2007) 363–370. doi:10.1093/mutage/gem027.

[45] M. Jokar, G.A. Pedersen, K. Loeschner, Six open questions about the migration of engineered nano-objects from polymer-based food-contact materials: a review, Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 34 (2017) 434–450. doi:10.1080/19440049.2016.1271462.

[46] Echegoyen Y, Rodríguez S, Nerín C. Nanoclay migration from food packaging materials. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2016. 33(3):530-9.

[47] J.Y. Huang, X. Li, W. Zhou, Safety assessment of nanocomposite for food packaging application, Trends Food Sci. Technol. 45 (2015) 187–199. doi:10.1016/j.tifs.2015.07.002.

[48] European Commission Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Official Journal of the European Union L 342. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1223&from=EN

[49] J. Rydz, G. Adamus, K. Wolna-Stypka, A. Marcinkowski, M. Misiurska-Marczak, M.M. Kowalczuk, Degradation of polylactide in paraffin and selected protic media, Polym. Degrad. Stab. 98 (2013) 316–324. doi:10.1016/j.polymdegradstab.2012.09.010.

[50] F. Iñiguez-Franco, R. Auras, G. Burgess, D. Holmes, X. Fang, M. Rubino, H. Soto-Valdez, Concurrent solvent induced crystallization and hydrolytic degradation of PLA by water-ethanol solutions, Polymer (Guildf). 99 (2016) 315–323. doi:10.1016/j.polymer.2016.07.018.

[51] European Commission regulation (EC) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0010&from=EN

[52] ISO 10993-5:2009 Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity. International Organization for Standardization. https://www.iso.org/obp/ui/#iso:std:iso:10993:-5:ed-3:v1:en

[53] 51. BS EN 1186-1:2002 Materials and articles in contact with foodstuffs — Plastics — Part 1: Guide to the selection of conditions and test methods for overall migration. British-Adopted European Standard. http://img.21food.cn/img/biaozhun/20090815/187/11183625.pdf

[54] 52. OECD (2015), Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264242845-en (accessed 04 December 2018).

[55] A. Coquette, N. Berna, A. Vandenbosch, M. Rosdy, B. De Wever, Y. Poumay, Analysis of interleukin-1α (IL-1α) and interleukin-8 (IL-8) expression and release in in vitro reconstructed human epidermis for the prediction of in vivo skin irritation and/or sensitization, Toxicol. Vitr. 17 (2003) 311–321. doi:10.1016/S0887-2333(03)00019-5.

[56] B. Schmidt, V. Katiyar, L. Plackett, E.H. Larsen, N.Gerds, C. Bender Koch, J.H. Petersen. Migration of nanosized layered double hydroxide platelets from polylactide nanocomposite films, Food Addit. Contam. 28 (2011) 956–966. doi: 10.1080/19440049.2011.572927.

[57] P. Šimon, Q. Chaudhry, D. Bakoš, Migration of engineered nanoparticles from polymer packaging to food - A physicochemical view, J. Food Nutr. Res. 47 (2008) 105–113.

[58] N. Herrera, A.A. Singh, A.M. Salaberria, J. Labidi, A.P. Mathew, K. Oksman, Triethyl citrate (TEC) as a dispersing aid in polylactic acid/chitin nanocomposites prepared via liquid-assisted extrusion, Polymers (Basel). 9 (2017). doi:10.3390/polym9090406.

[59] R.K. Shah, D.R. Paul, Organoclay degradation in melt processed polyethylene nanocomposites, Polymer (Guildf). 47 (2006) 4075–4084. doi:10.1016/j.polymer.2006.02.031.

[60] W. Xie, Z. Gao, W.P. Pan, D. Hunter, A. Singh, R. Vaia, Thermal degradation chemistry of alkyl quaternary ammonium Montmorillonite, Chem. Mater. 13 (2001) 2979–2990. doi:10.1021/cm010305s.

[61] T. V. Duncan, Release of engineered nanomaterials from polymer nanocomposites: The effect of matrix degradation, ACS Appl. Mater. Interfaces. 7 (2015) 20–39. doi:10.1021/am5062757.

[62] M. Mutsuga, Y. Kawamura, K. Tanamoto, Migration of lactic acid, lactide and oligomers from polylactide food-contact materials, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 25 (2008) 1283–1290. doi: 10.1080/02652030802017529.

[63] A. Nasiri, S. Peyron, E. Gastaldi, N. Gontard, Effect of nanoclay on the transfer properties of immanent additives in food packages, J. Mater. Sci. 51 (2016) 9732–9748. doi:10.1007/s10853-016-0208-x.

[64] S. Maisanaba, S. Pichardo, M. Jordá-Beneyto, S. Aucejo, A.M. Cameán, Á. Jos, Cytotoxicity and mutagenicity studies on migration extracts from nanocomposites with potential use in food packaging, Food Chem. Toxicol. 66 (2014) 366–372. doi:10.1016/j.fct.2014.02.011.

[65] Schmidt B., Petersen JH, Bender Koch, C, Plackett, D., Johansen, NR, Katiyar, V, Larsen EH. Combining asymmetrical flow field-flow fractionation with light-scattering and inductively coupled plasma mass spectrometric detection for characterization of nanoclay used in biopolymer nanocomposites. Food Addit. Contam. 2009. 26:1619–1627.

[66] C.A. Diaz, Y. Xia, M. Rubino, R. Auras, K. Jayaraman, J. Hotchkiss, Fluorescent labeling and tracking of nanoclay, Nanoscale. 5 (2013) 164–168. doi:10.1039/c2nr32978f.

[67] R.E. Conn, J.J. Kolstad, J.F. Borzelleca, D.S. Dixler, L.J. Filer, B.N. Ladu, M.W. Pariza, Safety assessment of polylactide (PLA) for use as a food-contact polymer, Food Chem. Toxicol. 33 (1995) 273–283. doi:10.1016/0278-6915(94)00145-E.

[68] B. Tyler, D. Gullotti, A. Mangraviti, T. Utsuki, H. Brem, Polylactic acid (PLA) controlled delivery carriers for biomedical applications, Adv. Drug Deliv. Rev. 107 (2016) 163–175. doi:10.1016/j.addr.2016.06.018.

[69] K.M. Zia, M. Zuber, M. Barikani, R. Hussain, T. Jamil, S. Anjum, Cytotoxicity and mechanical behavior of chitin-bentonite clay based polyurethane bio-nanocomposites, Int. J. Biol. Macromol. 49 (2011) 1131–1136. doi:10.1016/j.ijbiomac.2011.09.010.

[70] S. Maisanaba, D. Gutiérrez-Praena, S. Pichardo, F.J. Moreno, M. Jordá, A.M. Cameán, S. Aucejo, Á. Jos, Toxic effects of a modified montmorillonite clay on the human intestinal cell line Caco-2, J. Appl. Toxicol. 34 (2014) 714–725. doi:10.1002/jat.2945.

[71] R. Nigmatullin, F. Gao, V. Konovalova, Polymer-layered silicate nanocomposites in the design of antimicrobial materials, J. Mater. Sci. 43 (2008) 5728–5733. doi:10.1007/s10853-008-2879-4.