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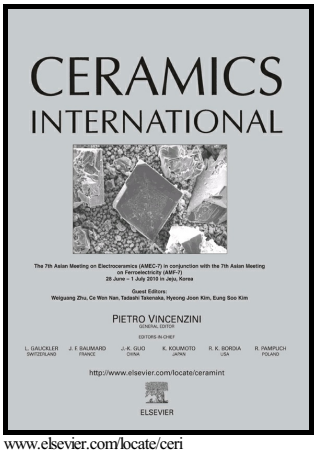

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PII: S0272-8842(16)31172-5
DOI: http://dx.doi.org/10.1016/j.ceramint.2016.07.107
Reference: CERI13332

To appear in: *Ceramics International*

Received date: 27 June 2016
Accepted date: 15 July 2016

Cite this article as: A (Thanos) Bourtsalas, Rainer Detsch, Aldo R. Boccaccin and Christopher Cheeseman, Initial studies on the cytotoxicity of ceramic prepared from dry discharge incinerator bottom ash dust, *Ceramics Internationa* http://dx.doi.org/10.1016/j.ceramint.2016.07.107

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Initial studies on the cytotoxicity of ceramics prepared from dry discharge incinerator bottom ash dust

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Abstract

The dust generated from a dry discharge ash handling system at a waste to energy (WtE) facility has been processed and sintered to produce ceramic samples. Preliminary cell culture studies have been completed to assess the potential cytotoxicity of the IBA ceramics prepared from dry discharge dust. The tests have used mouse embryonic fibroblast (MEF) cells. Results indicate inhibition of cell adhesion by the IBA ceramic surface. However the samples were covered by cells and these expressed similar behaviour in the context of morphology and vitality to control cells on glass samples. The work demonstrates that fine IBA dust from dry discharge systems can be formed into ceramics and that biocompatibility testing has an important role in defining potential suitability for use of these types of waste-derived ceramics.

Keywords: *A. Incinerator bottom ash; B. Energy-from-Waste; C. Resource efficiency; D. Cytotoxicity; E. Up-cycling*

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1. Introduction

Combustion of residual MSW produces incinerator bottom ash (IBA), a heterogeneous mix of materials that includes concrete, ceramic, glass, brick, mixed fused materials, and ferrous (~10 wt.%) and non-ferrous metals (~1–3 wt.%), mainly copper and aluminium. IBA is typically ~25 to 30 wt. % of the total input residual MSW to a WtE facility. IBA can be either wet or dry discharged from the combustion chamber. Dry IBA discharge systems produce 10 to 20% less IBA by weight because of the lower water content. Dry discharge is also reported to improve metal recovery rates. This typically involves using standard drum or flat screen decks with mesh sizes to sort different size fractions, with metal recovery using magnetic and eddy current separators. There is also a significant fraction of fine IBA dust generated by dry discharge systems. This has particle sizes typically less than 1 mm and currently it is not reused. Normal practice in Switzerland where dry discharge systems are operating is to mix the dust with air pollution control (APC) residues and stabilise using Portland cement, prior to disposal in hazardous waste landfill, at significant cost [1].

Previous research has demonstrated that the dry discharge dust can be transformed into an inert material suitable for the production of hard, dense ceramics [2]. In order to increase the application potenteial of waste derived products, a complete characterisation of their possible cytotoxicity must be conducted [3]. The aim of this research was to assess the cytotoxicity of ceramics prepared from the fine particles of dry-discharged IBA dust. It reports, for the first time, initial studies on the cytotoxicity of ceramics formed from IBA.

2. Materials and methods

2.1 Characterisation of IBA dust from a dry discharge system

A representative 50 kg batch of the fine fraction of IBA was obtained from the Monthey EfW facility in Switzerland. The fine dust fraction represents about 10 wt. % of the total IBA produced at the plant.

X-ray fluorescence was used to characterise the chemical composition of the fine IBA, which consists of predominantly silicate based minerals, with approximately 75% w/w consisting of Si, Ca, Fe and Al oxides. The moisture content of the as-received fine IBA determined from the weight loss at 105 °C, was low, as it had not been quenched into water, unlike traditional wet discharged IBA. The dry discharged fine IBA resulted in a pH between 11.5 and 12.5 when placed in water, due to the leaching of alkali and alkali-earth elements into solution [2]. X-ray diffraction (XRD) was used to determine the crystalline phases in the milled powder. The major crystalline phases present were quartz (SiO_2), calcite ($CaCO_3$), gehlenite ($Ca_2Al_2SiO_7$) and hematite (Fe_2O_3) [2]. The effect of temperature on mass loss was investigated using thermogravimetric analysis. Samples indicated a 16% weight loss when heated to 1000

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°C. Significant weight loss occurs between 550 and 850 °C due to decomposition of calcite ($CaCO_3$) to CaO and CO_2 and the decomposition of other volatile phases [2].

2.2 Cell culture tests on sintered ceramic samples

Previous research had demonstrated that the addition of 20 wt.% of recycled soda lime silica glass significantly improved liquid phase sintering of the fine IBA dust [2]. The dry discharged IBA dust can be processed to produce a ceramic with similar but generally improved properties compared to commercially available terracotta ceramics. Calcining the samples at 1080 °C has resulted in the decomposition of volatile compounds and the production of an inert powder, and subsequent pressing and sintering at 1080 °C produces ceramic microstructures with polished surfaces to a 1µm finish as shown in Figure 1 [2].

In order to assess the cell response to IBA ceramics, mouse embryonic fibroblast (MEF) cells were used, as described elsewhere [4]. MEF cells were seeded at a density of 100,000 cells/sample, cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco®, Germany) supplemented with 10% (v/v) fetal calf serum (FCS) (Sigma-Aldrich, Germany) and 1% (v/v) antibiotic-antimycotic (Gibco®, Germany) and incubated in a humidified atmosphere of 95% relative humidity and 5% CO_2, at 37°C.

After incubation for 24 hours, cell viability of seeded MEFs on IBA ceramic disc samples was assessed through the enzymatic conversion of tetrazolium salt (WST-8 assay kit, Sigma Aldrich, Germany) after 24 hours of cultivation. Culture media was completely removed from the incubated films and subsequently 1 %(v/v) WST-8 assay kit containing culture medium was added and incubated for 2 hours. 100 µl of supernatant from each sample was transferred into a well of a 96 well-plate and the absorbance measured at 450 nm with a micro-plate reader (PHOmo, Autobio Labtec Instruments Co. Ltd. China).

Staining of the viable cells was performed with calcein acetoxymethyl ester (CalceinAM) and nuclei were visualized by nucleic acid stain (Hoechst).

To investigate the cell morphology, cells were fixed with 4% buffered paraformaldehyde and permeabilized with 0.2% Triton X-100 (Sigma-Aldrich, Munich, Germany) in PBS. Subsequently, cells were stained with rhodamine phalloidin, which selectively binds F-actin. Nuclei were visualised using the nucleic acid stain SYTOX.

3. Results and discussion

The viability of MEF cells on dry discharged IBA ceramics compared to the cell culture dish and to cover glass over 24 hours cultivation period was investigated by WST-8 assay. As shown in Fig

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ures 2 and 3, the viability of MEF cells on IBA samples was significantly reduced (approx. 50 %) compared to that on PS-Ref (100 %) and cover glass (110 %) after 24 hours cultivation.

To visualize the viability and morphology of living cells fluorescence staining was performed by using calcein AM that stains the cytoplasm of living cells. Cell shape integrity of the cell membrane and distribution of cells on the samples as well as density of the cell layer, the hallmarks of normal living cell equilibrium, were also evaluated by calcein staining. In order to assess the integrity of the nucleus, a nucleic acid staining was performed using DAPI. As shown in Figures 3 and 4, MEF cells on IBA samples expressed similar cell behaviour in the context of morphology and vitality compared to PS-Ref and cover glass [4].

As all investigated sample surfaces were covered by MEF cells (Fig. 4), it is interesting to know how the cells adhere to these surfaces. Therefore, actin cytoskeleton staining with rhodamine phalloidin was applied to study cell adhesion properties. As shown in Figure 4, the cytoskeleton in MEF cells is expressed and the orientation of stress fibbers in fibroblasts grown on PS-Ref and on cover glass is clearly visible after 24 hours of cultivation. In contrast, the formation of the cytoskeleton in MEF cells is inhibited by the direct contact to the IBA surface. The preliminary direct cell culture study carried out on IBA samples indicated that the material induces inhibition of cell adhesion, which may be also an effect of surface roughness. Nevertheless, the samples were covered by MEF cells and cells expressed similar cell behaviour in the context of morphology and vitality (see figure 4) compared to cover glass, a compatible material. More investigations are required to characterise the potential toxicity of the samples (and their degradation products) in realistic biological environments.

4. Conclusions

The fine fraction of IBA dust generated by a dry discharge IBA processing system can be processed to produce ceramics containing pyroxene group minerals. The processing transforms the crystalline phases present and allow pre-sintering volatilisation of gas generating phases. The cytotoxicity of the IBA dust ceramics has been investigated and compared to the cytotoxicity of glass, using mouse embryonic fibroblast (MEF) cells. This shows inhibition of cell adhesion by the IBA dust ceramic surface, although samples were covered by cells that had the same morphology and vitality as control cells on glass samples. More research data is needed in different environments to fully characterize the potential cytotoxicity of these new, fine IBA dust derived ceramic samples.

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This research was funded by the Global Waste to Energy Research and Technology Council (WTERT), Wheelabrator Technologies Inc., Martin Gmbh., and a Dixon Scholarship provided by Imperial College London.

References

[1] Dubois M. Towards a coherent European approach for taxation of combustible waste, Waste Manag., 33 (8), 1776-1783 (2013).

[2] Bourtsalas A., Vandeperre L., Grimes S., Themelis N., Koralewska R., Cheeseman C. Properties of ceramics prepared using dry discharged waste to energy bottom ash dust. Waste Management & Research, 1-11 (2015).

[3] Rozenstrauha I., Lodins E., Krage L., Drille M., Balode M., Putna I., Filipenkov V., Chinnam R.K., Boccaccini A.R. Functional properties of glass–ceramic composites containing industrial inorganic waste and evaluation of their biological compatibility. Ceramics International 39(7), 8007-8014 (2013).

[4] Ponsot, I. M. M. M., Pontikes, Y., Baldi, G., Chinnam, R. K., Detsch, R., Boccaccini, A. R., Bernardo, E., Magnetic glass ceramics by sintering of borosilicate glass and inorganic waste, Materials 7, 5565-5580 (2014).

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Figure 1. Optical microscope image of ceramic samples containing dry discharged fine IBA dust and 20 wt. % glass and produced with optimum conditions (i.e. wet ball milling for 24 hours, calcining and sintering at 1080 °C). Prior to examination samples were polished to a 1μm finish.

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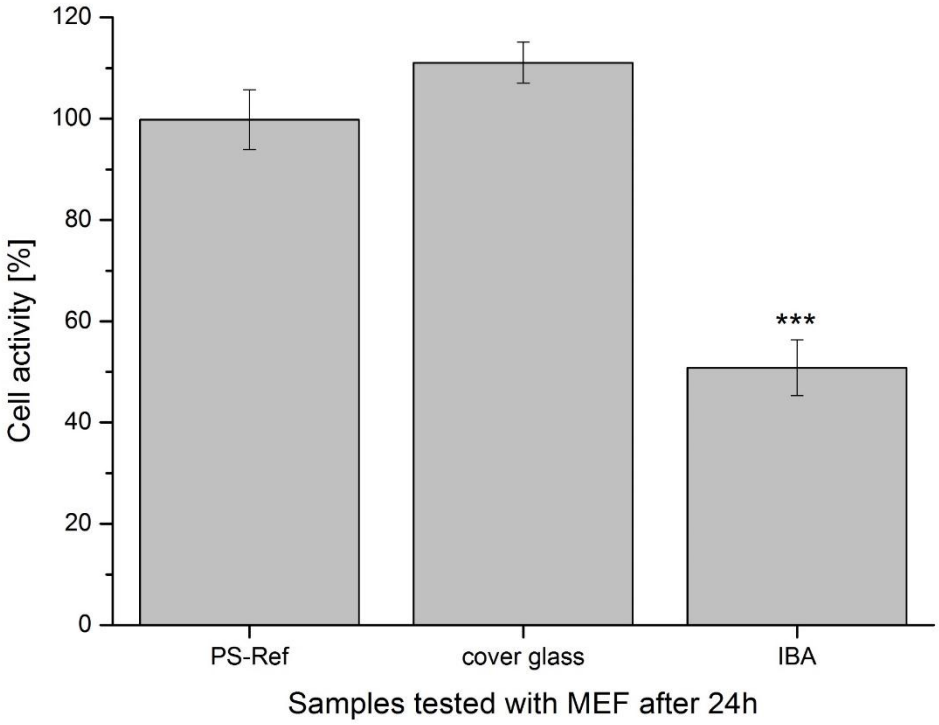

Figure 2. Viability of MEF cells on dry discharged IBA samples compared to the cell culture dish and to cover glass over 24 hours cultivation period. The viability of MEF cells on IBA samples was significantly reduced (approx. 50 %) compared to that on PS-Ref (100 %) and cover glass (110 %) after 24 hours cultivation

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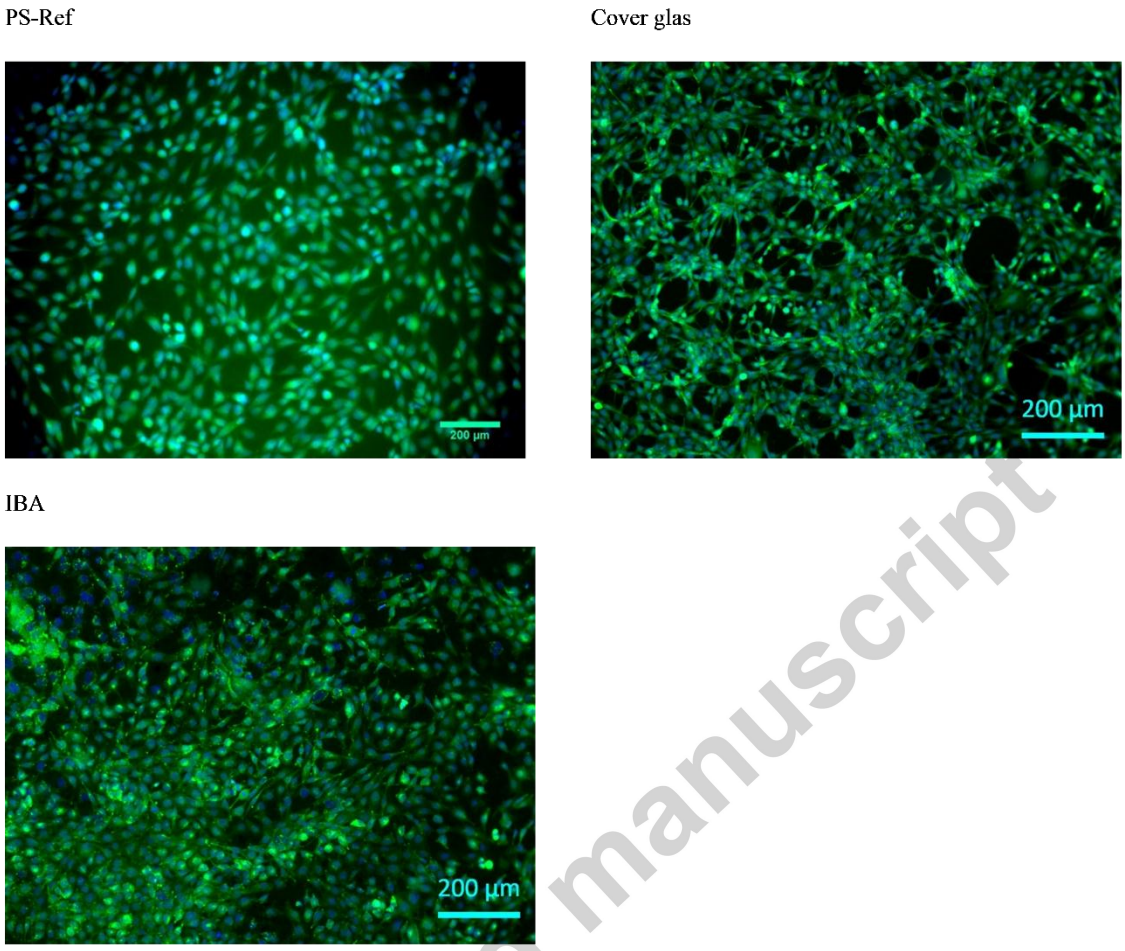

Figure 3. Morphology and vitality of MEF cells on IBA samples compared to PS-Ref and cover glass. MEF cells on IBA samples expressed similar cell behaviour in the context of morphology and vitality compared to PS-Ref and cover glass.

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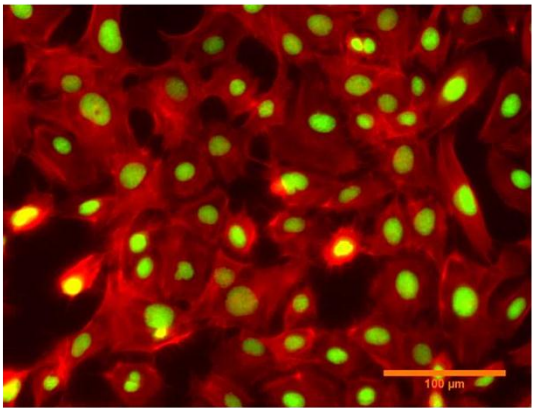
PS-Ref



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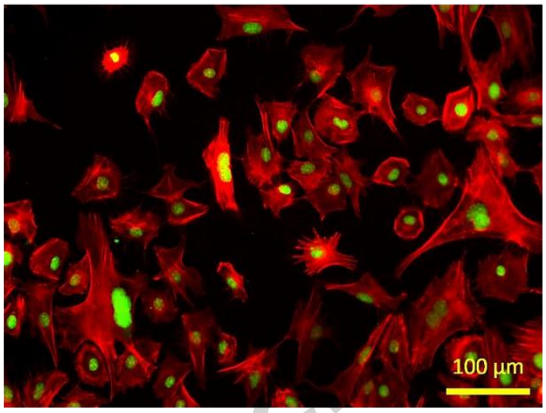

IBA

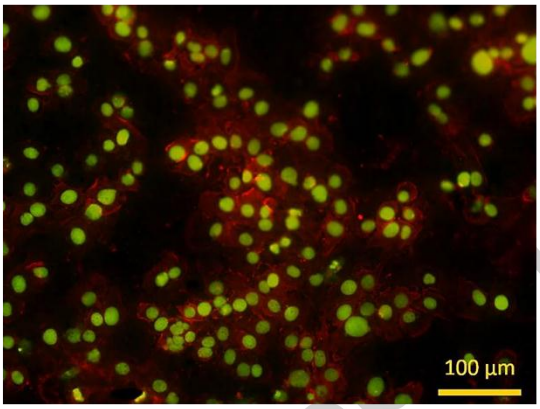

Figure 4. Images of cytoskeleton and orientation of stress fibbers in fibroblasts grown in the examined materials after 24 hours of cultivation. MEF cells grown on PS-Ref and on cover glass is clearly visible after 24 hours of cultivation. In contrast, the formation of the cytoskeleton in MEF cells is inhibited by the direct contact to the IBA surface.