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Direct reuse of electronic plastic scraps from computer monitor and keyboard to direct stem cell growth and differentiation

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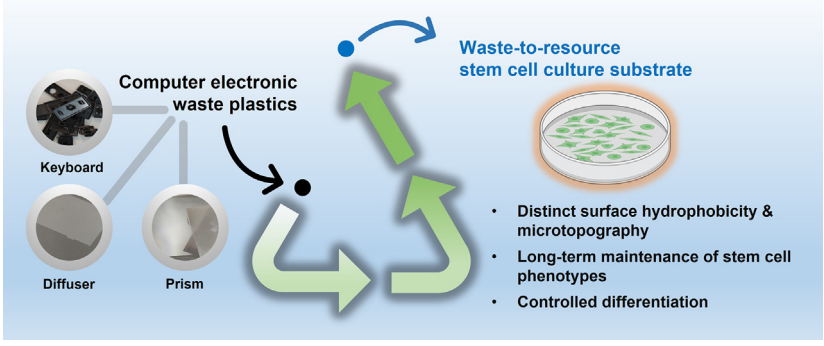
HIGHLIGHTS

- Innovative reuse of discarded E-plastics as cell culture substrate is presented.
- Leaching of metal additives is negligible under physiological conditions.
- E-plastics can support long-term self-renewability of human mesenchymal stem cells.
- E-plastics surface properties profoundly impact stem cells adhesion and differentiation.

GRAPHICAL ABSTRACT



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ABSTRACT

Reuse of electronic wastes is a critical aspect for a more sustainable circular economy as it provides the simplest and most direct route to extend the lifespan of non-renewable resources. Herein, the distinctive surface and micro topographical features of computer electronic-plastic (E-plastic) scraps were unconventionally repurposed as a substrate material to guide the growth and differentiation of human adipose-derived mesenchymal stem cells (ADSCs). Specifically, the E-plastics were scavenged from discarded computer components such as light diffuser plate (polyacrylates), prismatic sheet (polyethylene terephthalate), and keyboards (acrylonitrile butadiene styrene) were cleaned, sterilized, and systematically characterized to determine the identity of the plastics, chemical constituents, surface features, and leaching characteristics. Multiparametric analysis revealed that all the E-plastics could preserve stem-cell phenotype and maintain cell growth over 2 weeks, rivalling the performance of commercial tissue-culture treated plates as cell culture plastics. Interestingly, compared to commercial tissue-culture treated plastics and in a competitive adipogenic and osteogenic differentiation environment, ADSCs cultured on the keyboard and light diffuser plastics favoured bone cells formation while the grating-like microstructures of the prismatic sheet promoted fat cells differentiation via the process of contact guidance. Our findings point to the real possibility of utilizing discarded computer plastics as a "waste-to-resource" material to programme stem cell fate without further processing nor biochemical modification, thus providing an innovative second-life option for E-plastics from personal computers.

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1. Introduction

Our relentless pursuit for economic growth and newer electronic goods has generated a vast number of electronic wastes (E-waste) (Shaikh et al., 2020). In 2019 alone, the global production of E-waste was estimated to be 53.6 Mt. and it is expected to grow to a striking 74.7 Mt. by 2030 (Vanessa Forti et al., 2020). Plastics are integral component of electronics and accounts for 20% of E-waste by weight (Sahajwalla and Gaikwad, 2018). The current documented recycling rates of E-plastics are thought to be 17.4% at the global level (Vanessa Forti et al., 2020) with the remaining uncaptured E-plastic waste stream mostly incinerated and/or disposed in landfills. At least 15 different types of electronic plastics (E-plastics) such as a*crylonitrile butadiene styrene* (ABS), *poly*(*methyl methacrylate*) (PMMA), *poly*(*vinyl chloride*) (PVC), and *polystyrene* (PS), among other highly recyclable plastics have been documented (Buekens and Yang, 2014). Nonetheless, there is a limit as to how many times the plastics can be recycled as the mechanochemical properties of the recycled plastic can decrease significantly as a function of recycling cycles (Alhazmi et al., 2021). Additionally, the recycling of E-plastics is hampered by contamination and the complex compositional make-up of the plastic waste streams. Toxic metal-based colorants, stabiliser, catalysts, and brominated flame retardants (BFRs) were detected and measured in E-plastics at several orders of magnitude higher than the currently permissible levels and may get released into the environment if not disposed properly (Hahladakis et al., 2018; Singh et al., 2020). These toxic components can exert a broad spectrum of negative impacts on the environment and human health. In fact, a substantial number of recycled plastic products were found to contain toxic polybrominated diphenyl esters (PBDEs) and other BFRs (Gallen et al., 2014). Regardless, it is evident that E-plastics cannot be recycled indefinitely using the conventional mechanical and chemical pathways, and alternate waste management solutions should be sought to enable a holistic circular economy of E-plastics.

On a global scale, the demand for innovative solutions to minimise CO_2 emissions and waste valorisation is of increasing interest. Among the top priorities of the zero-waste hierarchy by the European Commission's Waste Framework Directive is to promote the reuse of plastic wastes (Billiet and Trenor, 2020). Different from recycling, the reuse option offers the shortest path with minimum expenditure of energy to return the E-plastics into the "use" phase of the plastic value chain via pathways such as refurbish, remanufacture, or repurpose (Reike et al., 2018). Exercising the reuse option enables the immediate extension of the E-plastics lifespan and minimizes environmental pollution as a consequence of indiscriminate landfill disposal. Furthermore, by reusing the discarded scraps attuned for another function, the materials get a specific new life cycle. For instance, several innovative reuse of E-plastics for 3D printing (Gaikwad et al., 2018; Mikula et al., 2020), construction (Senthil Kumar and Baskar, 2015; Dombe et al., 2020), and the automotive industry (Sahajwalla and Gaikwad, 2018) have emerged in recent years.

Owing to its abundance, ease of acquisition, excellent self-renewable and differentiation properties, adipose-derived mesenchymal stem cells (ADSCs) play a key role in the field of regenerative and reconstructive medicine. Numerous studies have shown the clinical utility of ADSCs-based therapies for the treatment of diseases such as neurological disorders, cardiac ischemia, diabetes, and bone and cartilage diseases (Semenova et al., 2018; Dompe et al., 2019; Mazini et al., 2021). Moreover, ADSCs are broadly employed in basic scientific research including tissue modulation, tissue engineering, and development and drug testing, etc. (Zhang et al., 2020). In most cases, the cells are conventionally grown on smooth and sterile tissue culture plate made primarily using polystyrene (polystyrene TCP). However, biology is more than just a monotonous and flat terrain and it has been established that surface properties such as wettability, chemistry, topography, stiffness can profoundly influence fundamental cellular processes such as adhesion, spreading, proliferation, and differentiation (Amani et al., 2019; Paiva et al., 2020).

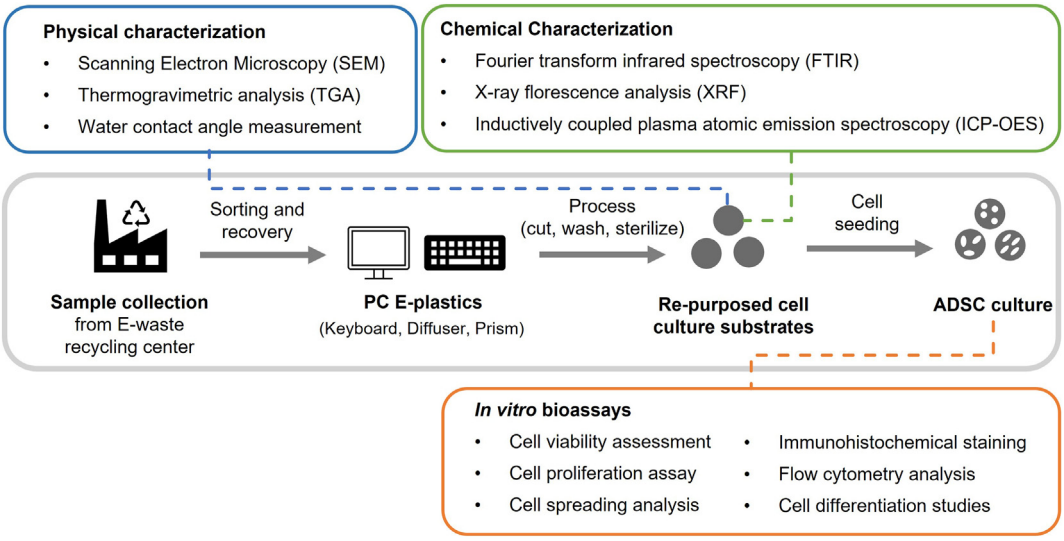
In this study, we took a leaf out of the biomaterials playbook and repurposed E-plastics from personal computers (PC) as second life cell culture plastics for stem cells culture. Our approach is in part inspired by our earlier study, where we showed that E-plastics such as the prism, keyboard, diffuser, and screen protector can support the long-term growth of several human cell lines without any significant cytotoxic effects (Shi et al., 2020). Furthermore, the generation of research laboratory plastic wastes (including cell culture plastics) is substantial, which was estimated to be around 5.5 million tonnes in 2014 alone (Urbina et al., 2015). To put things into perspective, this figure significantly dwarfs the reported annual 18,500 t plastics flow 3D printing applications (Li, 2017). Therefore, the potential of E-plastics reuse for cell culture application is posited to be at least on par with 3D printing. Specifically, we hypothesize E-plastics scavenged from the discarded PC with distinct surface properties, namely, keyboard, light guide plate, screen diffuser could potentially be exploited to direct the growth and behaviour of ADSCs. Three representative E-plastics namely, keyboard, light guide plate, screen diffuser was examined. The screen diffuser sheets in the liquid-crystal displays (LCDs) possess highly aligned prismatic ridges which could potentially guide cellular alignment (Leclech and Villard, 2020; Shi et al., 2020). The diffuser sheets have smooth and flexible surfaces, while the surface of the keyboard casings is mechanically tough and textured. The identity, chemical composition, surface properties of the E-plastics were systematically investigated. Our proof-of-concept studies revealed that the E-plastics not only supported the long-term growth of ADSCs, but also retained the multilineage differentiation potential of the cells as indicated by robust expression of STRO-1 and CD166 stemness markers (Garcia et al., 2016). Intriguingly, using polystyrene TCP as a benchmark, osteogenic differentiation of ADSCs was enhanced on keyboard and diffuser E-plastics, while the prism sheet favours adipogenesis. Our findings unveil a first-of-a-kind and previously untapped potential of repurposing E-plastics as specialty substrates to renew and coax lineage-specific differentiation of mesenchymal stem cells.

2. Material and methods

2.1. Materials and reagents

The E-plastics consisted of scraps from keyboards and LCDs were curated from Virogreen (Singapore) PTE Ltd. The samples were transported to the laboratory and manually dismantled using a screwdriver and penknife. In all cases, the electronics board can be easily separated from the plastics casing or components, thereby minimising cross-contamination of the metallic parts. The key buttons from the keyboard can be easily dislodged with the use of a flathead screwdriver. Meanwhile, the non-adhesive prism and diffuser sheets located in between the backlit and liquid crystals of the LCD screen were separated by hand. The plastic parts were subsequently cut into a circular shape (radius = 11 mm), and washed by double distilled water, followed by sonication and sterilization in 70% ethanol. The samples were air-dried in biological safety cabinet and stored under sterile conditions for further biological investigations. ASC52telo, hTERT immortalized adipose-derived mesenchymal stem cells (ADSCs, SCRC-4000) were purchased from American Type Culture Collection (ATCC). ADSCs were maintained in mesenchymal stem cell basal medium with the addition of a mesenchymal stem cell growth kit (PCS-500-030 and PCS-500-040). Mouse anti-stro1 antibody (Stro-1) and rabbit anti-CD166 antibody (CD166) were purchased from Abcam. Insulin, transferrin, sodium selenite, goat anti-mouse IgG (H + L) highly cross-adsorbed secondary antibody, Alexa fluor plus 488, and chicken anti-rabbit IgG (H + L) cross-adsorbed secondary antibody, Alexa fluor 594 were purchased from ThermoFisher Scientific. Dexamethasone, ascorbic acid, β-glycerophosphate, indomethacin, and 3-isobutyl-1-methylxantine (IBMX) were purchased from Sigma-Aldrich.

The overall experimental design and workflow is illustrated in Schematic 1.



Schematic 1. Experimental design, workflow, and techniques employed in this study.

2.2. Fourier transform infrared spectroscopy (FTIR)

The E-plastics were evaluated using an FTIR spectrometer (Perkin Elmer Frontier), to identify the types of polymers and inorganic/organic additives (Subramanian, 2013). FTIR is an adaptable non-destructive vibrational spectroscopic technique that can determine the molecular and functional groups present in plastic polymers. FITR spectroscopy measures the infrared (IR) radiation absorbed by the plastic sample, thus enabling the study of its molecular composition. The E-plastics were cut into small pieces, and further grounded into a fine powder, and thoroughly blended with KBr. Subsequently, the mixtures were compacted into pellets for examination. The samples were scanned 16 times respectively at an absorption wavelength ranging from 400 to 4000 cm^{-1} at a resolution of 2 cm^{-1}.

2.3. Water contact angle measurements

Surface wettability was evaluated by the static water contact angle measurements. The contact angle was determined by the sessile drop method with optical contact angle measuring and contour analysis systems (DataPhysics Instruments, Germany). Ultra-pure water droplets of 0.25 μL were applied to the E-plastic samples. Measurements were carried out at constant conditions (25 °C and 60% humidity). The contact angle was calculated as an average of three measurements and was expressed as mean ± standard deviation (SD).

2.4. X-ray fluorescence (XRF) analysis

Elemental analysis was carried out using a modified handheld X-ray fluorescence analyzer (Vanta C Series Handheld XRF analyzer, Olympus), following the previously published protocol (Shi et al., 2020). The handheld XRF analyzer was pre-calibrated by the manufacturer for plastic analysis. For safety reasons, the XRF analyzer was stabilized by a field stand during measurements, and the plastic samples were placed on a silicon drift detector within a protective shield. Exposure duration of approximately 180–500 s was used for each analysis, including a dwell time of 60 s in the low energy range (30 kV and 66.67 μA to measure Cl, Cr, and Ti elements) and main energy range (50 kV and 40 μA: all remaining elements measurements). The generated X-ray spectra were automatically deconvoluted and semi-quantified to determine the dry weight elemental concentrations (parts per million, ppm).

2.5. Inductively coupled plasma atomic emission spectroscopy (ICP-OES)

The E-plastics samples including keyboard, prism, and diffuser sheets were incubated in ADSC basal media, DMEM, and competitive differentiation media respectively for 14 days. The volume ratio of sample to cell culture media is consistent with the ratio during cell culture and differentiation studies. Briefly, the samples were completely submerged into cell culture media in securely sealed centrifuge tubes at 37 °C. Two weeks later, the supernatants were collected, filtered (syringe filter, Millipore), and stored at 4 °C for future analysis via ICP-OES. The detection limit of the ICP-OES is 1 ppm. Polystyrene TCP were also analyzed by XRF and ICP-OES as control.

2.6. Cell culture and seeding

ADSCs were maintained in mesenchymal stem cell basal medium with the addition of a mesenchymal stem cell growth kit (PCS-500-030 and PCS-500-040). Fifty thousand (5×10^4) cells were seeded on each E-plastic and polystyrene TCP, and they were cultured at least 24 h before cell viability assay and differentiation study. Meanwhile, the ADSCs on E-plastics and polystyrene TCP were cultured in basal media for 2 weeks for Stro-1 and CD166 analysis. The cells were routinely replenished with fresh cell culture or differentiation medium every 3–4 days.

2.7. Cell viability and proliferation assay

5×10^4 ADSCs were seeded on the E-plastics respectively, and the cells were maintained in stem cell growth media. Stem cells viability was monitored using the PrestoBlue assay in accordance with earlier studies (Yang et al., 2019a, 2019b, Shi et al., 2020, Wang et al., 2020, Wang et al., 2021). Briefly, at predetermined time points (i.e., 1, 4, 7 days), the original cell culture media was removed from the E-plastic grown stem cells samples. Thereafter, 1 ml 10% Prestoblue (in basal medium) was added to the respective medium samples and mixed by pipetting. After 60 min of the incubation period, 200 μl of the reaction mix was pipetted directly into a 96 well plate and the reduced Prestoblue signal was determined at fluorescence excitation/emission maxima of 560/590 nm using a SpectraMax M2 microplate reader (Molecular Devices, USA). Cells cultured on conventional polystyrene TCP served as control.

2.8. Scanning electron microscope (SEM)

The original plastic samples of the keyboard, prism, and diffuser were sputter-coated with platinum and observed under thermionic SEM (JEOL 6360). The ADSCs seeded prism sheet at day 14 were fixed by 4% PBS buffered paraformaldehyde, and the cells were dehydrated by ethanol, and eventually dried in a critical point dryer (Tousimis Samdri-PVT-3D). The dried samples were sputter-coated by platinum and observed under thermionic SEM (JEOL 6360). All the samples were observed under 5KV (accelerating voltage).

2.9. Morphological analysis of ADSCs

The ADSCs on the prism, diffuser, keyboard, and polystyrene TCP samples were fixed on day 7 and 14 respectively. The samples were permeabilized by 0.1% Triton X-100 for 5 min and washed by 0.05% Tween-20/PBS three times. The permeabilized cells were stained by Alexa Fluor™ 594 Phalloidin (ThermoFisher scientific), and the nucleus was stained by Hoechst 33258, Pentahydrate (ThermoFisher scientific). The stained cells on E-plastics were observed under a fluorescence microscope (Carl Zeiss).

2.10. Stemness markers of ADSCs on E-plastics

The ADSCs were seeded on the e-plastics and cultured in ADSC basal medium for two weeks. Subsequently, the cells were detached, centrifuged, and fixed by ice-cold methanol, and the cells were spun down to remove methanol. For immunostaining, the cells were permeabilized by 0.1% Triton X-100 for 5 min and washed by 0.05% Tween-20/PBS three times, then the samples were blocked by 1% bovine serum albumin (BSA). One hour later, the samples were incubated in 1% BSA solution containing mouse monoclonal anti-STRO1 antibody (Abcam) and rabbit monoclonal anti-CD166 antibody (Abcam) overnight. Subsequently, the samples were washed by PBS, and then they were incubated with Goat anti-mouse IgG (H + L) highly cross-adsorbed secondary antibody, Alexa fluor plus 488, and chicken anti-rabbit IgG (H + L) cross-adsorbed secondary antibody, Alexa fluor 594 for 1 h, respectively. The samples were then eventually washed by PBS three times and resuspended in 1 ml PBS and analyzed by a fluorescence microscope (excitation/emission: 495/519 nm and 591/614 nm, respectively) and flow cytometer (Guava easyCyte HT).

2.11. Differentiation of ADSCs on E-plastics

The osteogenic media was prepared by adding 10% FBS, 0.01 μM dexamethasone, 50 μg/ml ascorbic acid, 10 mM sodium β-glycerophosphate, 1% Penicillin/Streptomycin to the experimental culture media. Adipogenic Induction Media, consisting of DMEM-High glucose, supplemented with 10% FBS, 1% Penicillin/Streptomycin, 1 μM dexamethasone, 1 μM indomethacin, 500 μM IBMX, 10 μg/ml insulin, 5.5 μg/ml transferring and 5 ng/ml sodium selenite. The competitive differentiation media were prepared by mixing adipogenic and osteogenic induction media at an equal volume ratio. Fifty thousand (5×10^4) cells were seeded on E-plastics and control, the cell-seeded samples were maintained in the mesenchymal stem cell basal medium (Section 2.6) for 2–3 days for cell accommodation, then the cells on the respective E-plastic were cultured in the competitive differentiation media for two weeks. To quantify the differentiation of ADSCs towards osteogenic and adipogenic lineages, the cells on each plastic sample at week1 and 2 were trypsinized, and the cells on each plastic sample were collected and resuspended in basal media, and then they were allowed to attach on polystyrene TCP in 4 h. Subsequently, the cells were fixed by 4% paraformaldehyde and stained by alizarin red and oil red individually. The stained cells from each sample were observed under the microscope, the alizarin red and oil red positive cells were counted, and the proportion of osteogenic and adipogenic differentiation of ADSCs was calculated and plotted.

2.12. Data analysis

All data were expressed as mean ± standard deviation. Statistical analysis was performed by pairwise comparison of experimental categories using a two-tailed, unpaired Student's *t*-test and multiple comparisons using single-factor analysis of variance (ANOVA) and post hoc Tukey tests, using SPSS Statistics version 22.0. A *p*-value of less than 0.05 is considered statistically significant.

3. Results and discussion

3.1. Characterization of E-plastics

Fig. 1A–C shows the pictographs of the discarded keyboard black pushbuttons, diffuser, and prism sheets obtained from the disposed liquid crystal displays (LCD) respectively. Both the keyboard and diffuser plastics presented a relatively flat and smooth surface under the SEM (Fig. 1D–E). Conversely, the prism surface was revealed to be composed of highly aligned prismatic ridges of height approximated to be around 23 μm and pitch length of 52 μm (Fig. 1F). Besides surface topographies, the wettability of a plastic surface (hydrophilicity and hydrophobicity) has also been highlighted as a critical regulator of protein adsorption and cell-material interaction (Song and Mano, 2013). Therefore, we performed the static water contact angle measurement to determine the wetting property of the E-plastic. Generally, a moderate hydrophobicity supports cellular adhesion and growth, while surfaces with extreme hydrophilicity (contact angle <5°) or hydrophobicity (contact angle >150°) present an interface that is thermodynamically unfavourable for cells to anchor onto the substrate (Law, 2015). This is because surfaces with exceptional hydrophilic or hydrophobic features can suppress serum protein adsorption, which is considered as an obligatory process to establish cell-substratum contact. Since cell spreading and division are always proceeded by cell adhesion, the inability for the adherent cells to "stick" to the material surface would be detrimental to cell health (Tay et al., 2011a, 2011b). In the case of the E-plastics, the measured water contact angles were 97.0 ± 1.6°, 81.7 ± 4.4°, and 60.0 ± 2.5° for the keyboard, diffuser, and prism groups respectively, which is well-within the cell-adhesive range (Fig. 1G).

Since the E-plastics were produced by different manufactures using untraceable raw materials worldwide, therefore, it is pivotal to characterize and identify their polymer composition. Fig. 1H shows the spectra of the corresponding E-plastics sample and the detected absorption peaks are summarised in Table S1. All FTIR spectra were cross-checked and verified with the KnowItAll IR Spectral Library as per the earlier report (Shi et al., 2020). Noteworthy, peak at 1602 cm^{-1} of the keyboard (Fig. 1H-I c) belongs to stretching vibration of C═C double bond from butadiene units, while the peak at 1494 cm^{-1} (Fig. 1H-I d) represents the stretching vibration of the aromatic ring from styrene of acrylonitrile butadiene styrene (ABS), while the characteristic absorption peak at 2238 cm^{-1} (Fig. 1H-I b) can be attributed to the C≡N bond within ABS polymer chain (Li et al., 2017). Conversely, the FTIR spectrum of the diffuser sheet (Fig. 1H-II b, c, and d) revealed several distinct absorption bands at 1720, 1158, and 1253 cm^{-1}, which correspond to the stretching vibration of carbonyl, ether, and epoxy groups of acrylic and methacrylic polymers respectively (Dzulkurnain et al., 2015). In the case of the prism sheet sample (Fig. 1H-III), the absorption peaks for C═O in the carbonyl group (**a**, 1713 cm^{-1}), and the C—O stretching modes in alkanoate ester (**b**, 1241 cm^{-1}) and alkoxy ether (**c**, 1094 cm^{-1}) were detected(Prasad et al., 2011). Meanwhile, the peaks at 980 and 835 cm^{-1} (Fig. 1H-III d, and e) correspond to Si-O-Si and Si-OH of glass (silica) respectively (Oh and Choi, 2010). Therefore, it was deduced that the prism sheet is made of glass-reinforced polyethylene terephthalate (PET).

Next, based on the RoHS and Directives of the European Parliament and the Council (2009/48/EC, 2011/65/EU, and 94/62/EC), XRF was employed to detect representative toxic compounds of interests such as

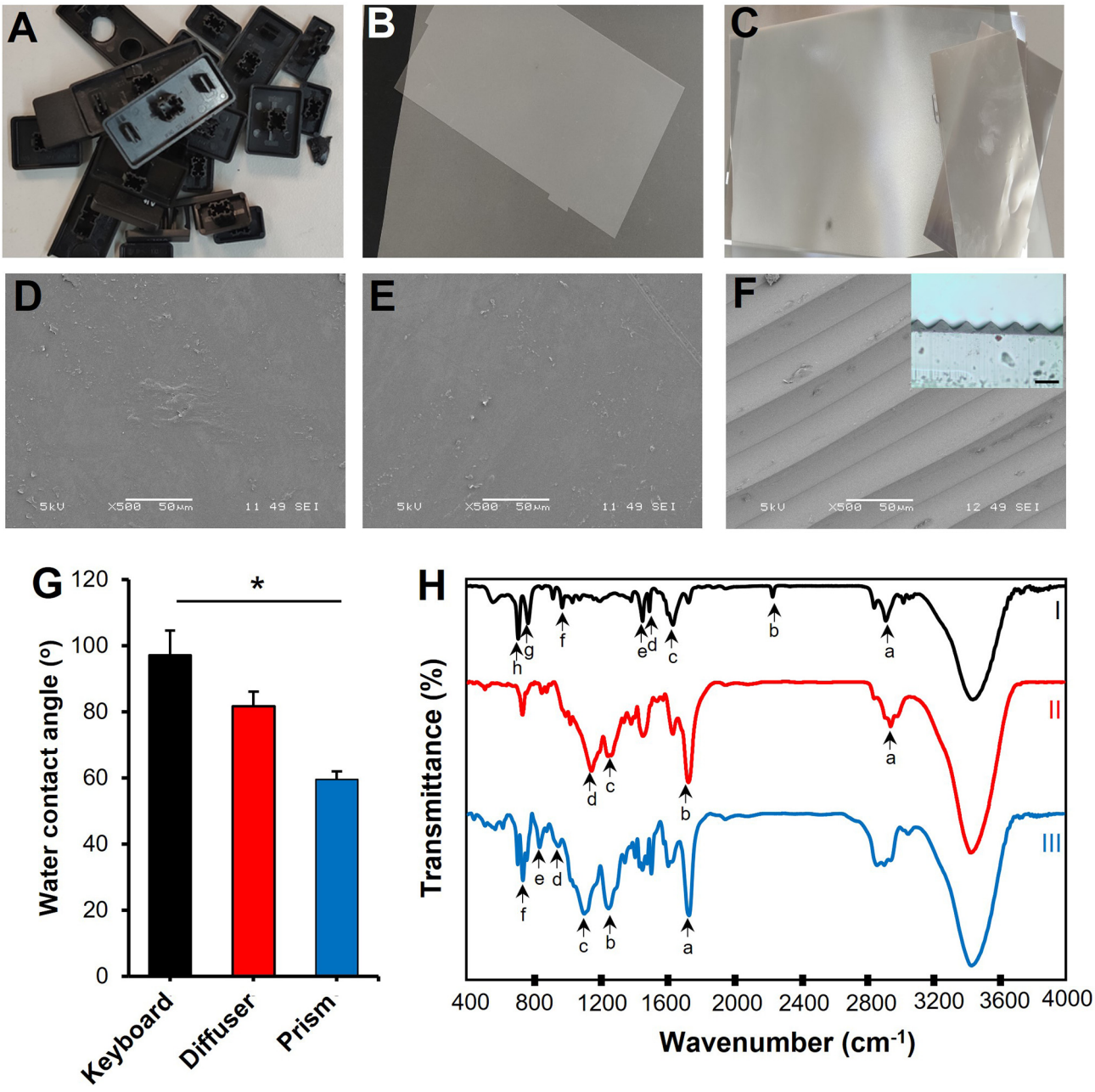


Fig. 1. Representative pictographs (A–C) and SEM images (D–F) of the keyboard, diffuser sheet, and prism sheet were employed in this study. (Insert: cross-section of prism sheet, scale bar: 50 μm). (G) Static water contact angle measurements of the E-plastics. (H) FTIR spectra of the keyboard (I), diffuser (II), and prism sheet (III).

metals, brominated flame retardants. The presence of these toxic additives may potentially hinder the reuse of these E-plastics as cell culture substrates. The evaluated elements included Chlorine (Cl), Chromium (Cr), Manganese (Mn), Cobalt (Co), Nickel (Ni), Arsenic (As), Selenium (Se), Zirconium (Zr), Molybdenum (Mo), Cadmium (Cd), Gold (Au), Mercury (Hg), Lead (Pb), Bismuth (Bi), Calcium (Ca), Titanium (Ti), Zinc (Zn), Bromine (Br), Antimony (Sb), Barium (Ba), Iron (Fe), Copper (Cu) and Tin (Sn). In general, the majority of the elements were undetectable in the E-plastics samples. However, as shown in Fig. 2, elements such as Ca, Ti, Zn, Br, Sb, and Ba were detected in the keyboard plastics. In addition, Fe, Cu, Br, Tin, Sn, Sb, and Ba were also found to be present in the prism sheet, while similar types of elements (except Br) were observed in diffuser sheets. Further scrutiny of the XRF data revealed that several of the elements were detected at levels that exceed the limit set forth by RoHS and Directives of the European Parliament and the Council (2009/48/EC, 2011/65/EU, and 94/62/EC) standards (green arrows). Notably, the Sb content in all three E-plastics is considerably high. This is not unexpected as Sb_2O_3 is commonly added to plastics as inorganic flame retardants to withstand high-temperature usage (Nakashima et al., 2012). With the proposed cell culture application in mind, we next examined the leaching characteristics of the E-plastics in cell culture media at 37 °C. However, long-term (2 weeks) immersion of the E-plastics samples in the cell culture medium did not result in any appreciable metal leaching, as the concentration of the various metal species (i.e., Ba, Ni, Ag, Mn, Cu, Sb, Cr, Sr, Zn, Cd, As and Ti, etc.) into the biological milieu were extremely low (less than 1 ppm) (data not shown). This observation is consistent with our earlier studies, which showed that the outward migration of metal ions from various E-plastics was <1 ppm (Mao et al., 2020; Shi et al., 2020) and not deemed to be cytotoxic (Banfalvi, 2011).

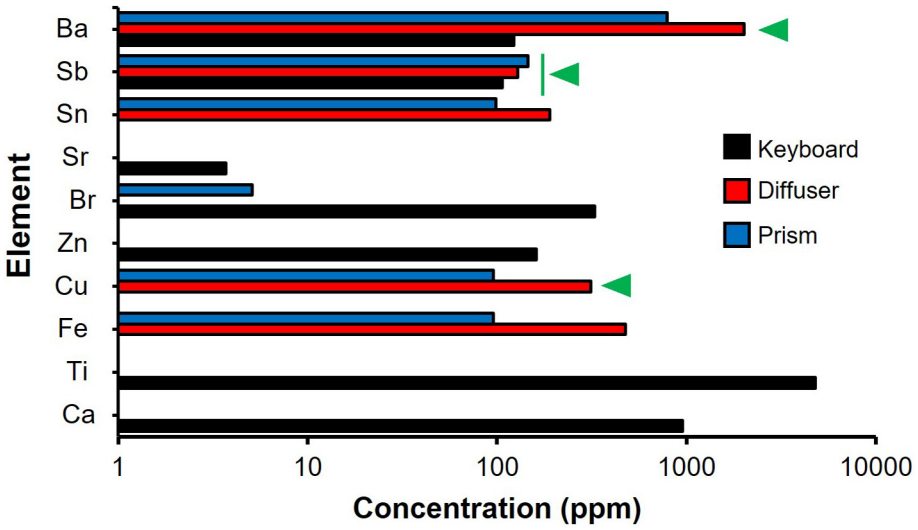


Fig. 2. XRF analysis of E-plastics. Green arrow indicates elements that are detected at levels that exceeds the restriction limits of hazardous substances in accordance to 2009/48/EC, 2011/65/EU and 94/62/EC. Threshold limits of various metal species: Cu (156 ppm), Zn (938 ppm), As (3.8 ppm), Sr (1125 ppm), Sn (3750 ppm), Sb (45 ppm), Ba (1125 ppm), Pb (13.5 ppm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Self-renewing capacity of ADSCs on E-plastics

ADSCs are non-specialized cells with the ability to self-renew and differentiate into cells of different tissue lineages, such as adipocytes and osteoblasts. ADSCs have emerged as one of the most widely utilized and studies stem cells due to their ease of retrieval and abundancy (Si et al., 2019). Besides its utility in cell-based therapies, ADSCs can also be applied as a powerful in vitro tool to advance our understanding of tissue development, establish physiologically relevant disease models to aid drug development, and elucidate important biological pathways for novel therapeutic interventions (Tay et al., 2013; Si et al., 2019; Yang et al., 2019a, 2019b). To fully exploit ADSCs, suitable cell culture surfaces and conditions need to be optimized to promote cell growth while maintaining the differentiation potential (stemness) of the stem cells. Therefore, we first examined the ability of the sterilized E-plastics to support the adhesion and proliferation of ADSCs using the resazurin-based PrestoBlue assay. Polystyrene TCP was employed as a control substrate to evaluate the suitability of the E-plastics for stem cell culture application. In agreement with the earlier data which shows limited metal ion leaching from the plastic samples, ADSCs displayed high viability (>95%) for all the E-plastics group over 1 week, at the level that is comparable to the polystyrene TCP control (Fig. 3). Furthermore, these results are consistent with the moderate wettability of the E-plastics (Fig. 1G), thereby permitting serum protein adsorption, cell attachment, and viability maintenance. This implies that the E-plastics are non-toxic and cytocompatible.

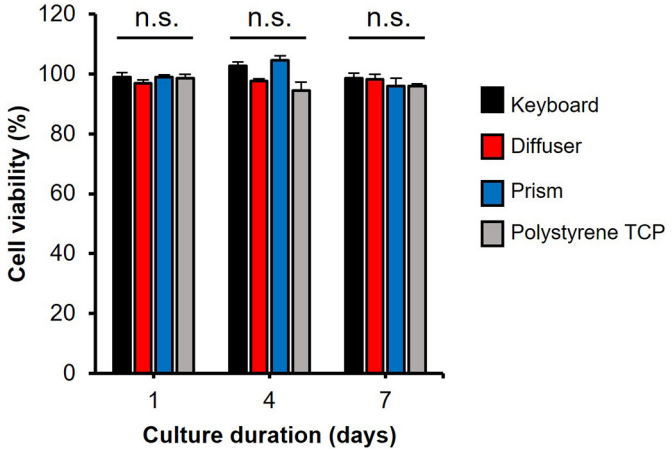


Fig. 3. Quantitative cell viability assessment of ADSCs grown on various E-plastics substrates. Polystyrene TCP serves as positive control. All data are represented as mean ± standard deviation from three experimental replicates. n.s. denotes no statistical difference between experimental groups relative to the polystyrene TCP control. Statistical significance is determined at $p < 0.05$.

Next, we ask if the long-term culture of ADSCs on the E-plastics could result in the loss of ADSCs' differentiation capacity. For this purpose, the cells were subjected to 2 weeks of culture on the E-plastics under basal growth condition and subsequently retrieved to probe for Stro-1 and CD166 expression using flow cytometry. Stro-1 and CD166 are well-established stemness markers of mesenchymal stem cells, and their expression is indicative of the cells' multipotency (Huang et al., 2013; Mildmay-White and Khan, 2017). As observed from the immunostaining images (Fig. 4A), ADSCs displayed robust concomitant expression of Stro-1 and CD166 after 2 weeks of culture on the respective E-plastics. Consistently, flow cytometry analysis revealed that both the Stro-1 and CD166 proteins were equally expressed on the ADSCs cultured on the keyboard, diffuser, prism, and polystyrene TCP individually. As can be seen in Fig. 4B, there is only one population in each graph, and the intensity of the two stemness markers is comparable among the four samples. Collectively, not only we show that the E-plastics are non-cytotoxic, but also encourage the long-term adhesion and expansion of ADSCs while maintaining the mesenchymal stem cells phenotype.

3.3. Osteogenic and adipogenic differentiation of ADSCs on E-plastics

To investigate the influence of E-plastics surface features in directing ASDCs lineage commitment, we examined osteogenesis and adipogenesis simultaneously in cultures using a mixed differentiation media containing both osteogenic and adipogenic factors (Fig. 5A). As shown in Fig. 5B, ADSCs exhibit distinct morphologies on the various substrates after 7 days of culture. Whereas ADSCs on the keyboard, diffuser, and polystyrene TCP displayed the typical flatten and spread morphology, cells grown on the prismatic sheet were highly elongated and aligned in the direction of the groove edge likely to be due to the "contact guidance" phenomenon (Tay et al., 2011a, 2011b). After 14 days of culture, there was a significant increase in the number of cells attached to the respective substrates. This observation clearly shows that the E-plastics can support the mitotic activity of the cells, which corroborates the

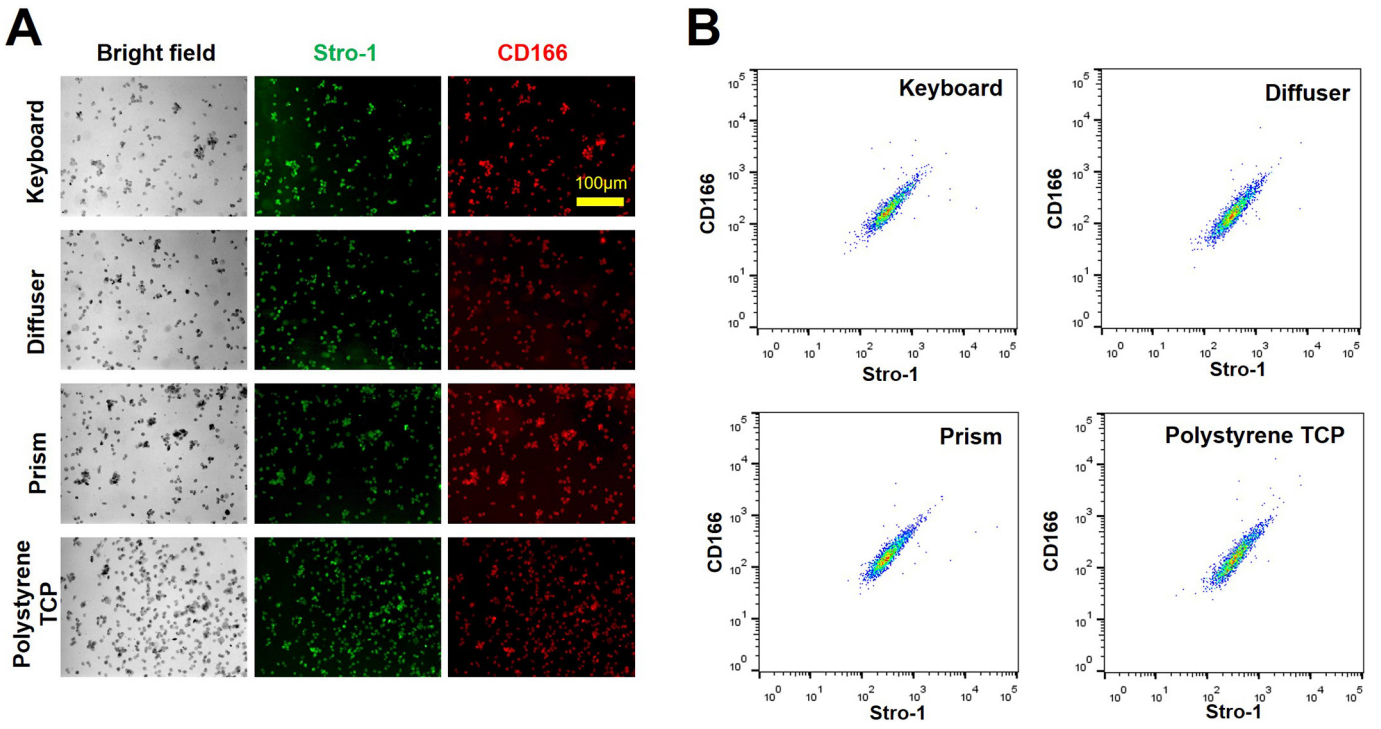


Fig. 4. (A) Representative bright field and fluorescence images of the recovered ADSCs counter-stained for Stro-1 (green) and CD166 (red) after 2 weeks of culture in basal medium on the respective substrates. Polystyrene TCP serves as the positive control. (B) Flow cytometry plot of ADSCs corresponding to the respective experimental group indicating Stro-1 (+) and CD166 (+) cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

viability data and highlights the cytocompatibility of the E-plastic wastes. The cells on the keyboard and diffuser E-plastic remained flattened while the cells on the polystyrene TCP surface appear to be bigger, slightly polarized with well-developed stress fibers. Conversely, cells grown on the prism sheet remain elongated and appear to congregate along the long edges of the prisms. Indeed, upon closer inspection under the SEM, ADSCs were found preferentially attaching and elongating along the apex of the prismatic ridges and only a few cells could be observed in the grooves (Fig. S1). This observation is consistent with an earlier study which showed that cell-substratum contacts were restricted to the tops of the ridges of silicon-based substrates with uniform grooves and ridges having pitch dimensions of 400–4000 nm (Karuri et al., 2004). A consequence of such adhesion pattern is that the cell spreading is severely limited spatially and can hinder the assembly of the cytoskeleton, as indicated by the diffuse/fragmented F-actin staining (Fig. 5B).

As a measurement of osteogenic and adipogenic differentiation, ADSCs were counter-stained with Alizarin Red S (for calcium) and Oil red O (for lipids), corresponding to makers of bone and fat cells, respectively (Fig. 5C–F). In general, all the tested E-plastics were observed to not only support osteogenesis and adipogenesis but also outperform the polystyrene TCP substrate in terms of differentiation efficiency. Specifically, after 2 weeks of culture, the total number of adipocytes and osteoblasts was less than 45% in the polystyrene TCP group, while the number of differentiated cells easily exceeds 80% on the E-plastics substrates. Unlike the smooth and flat surface presented on the polystyrene TCP, surface imperfections such as roughness, micro/nano-features that are inherent to the E-plastics may contribute to the enhancement of osteogenic and adipogenic differentiation (McBeath et al., 2004, Tay et al., 2013, Tay et al., 2015, Shukla et al., 2016, Nicolas, 2017a, 2017b, Khan et al., 2020). Furthermore, due to our competitive differentiation experimental design, the possibility of directing ADSCs to differentiate along a particular lineage on the various substrates can also be probed. Whereas the number of adipocytes and osteoblasts were comparable on the keyboard and prism E-plastics, there is a higher percentage of osteoblasts compared to adipocytes in the diffuser and polystyrene TCP groups after 7 days of culture. However, at the 14 days timepoint, all but the prism E-plastic displayed a tendency to favour osteogenesis. On average, the number of osteoblasts relative to the adipocytes was approximately 3 to 4-fold larger. The osteo-directing effect of the keyboard E-plastics was particularly evident with almost 90% of the cells stained positive with Alizarin Red S, followed by the diffuser (60%) and polystyrene TCP (40%) groups. From the materials standpoint, our findings are in agreement with earlier studies which showed that ABS (base material for keyboard E-plastic) could enhance osteogenic differentiation and regeneration (Schmelzer et al., 2016) (Helguero et al., 2017). On the other hand, extensive adipogenesis (~70%) was observed on the prism plastics. Although traces amount of leached-out additives probably can play a potential role to modulate the ADSCs behaviour, we believe that this possibility is low. This notion is supported by the extremely low levels (<1 ppm) of metals ions detected in the cell culture leachate, as well as the on-par metabolic activity (Fig. 3) and sustained expression of the stemness markers (Fig. 4) in the ADSCs grown on the E-plastics relative to the polystyrene TCP control.

Both Stro-1 and CD166 are primitive stemness markers of MSCs (McMurray et al., 2011) and their expression strongly suggest that the pro-longed contact made between the ADSCs and the E-plastics has negligible bearing in the absence of any soluble differentiation factors. Further mechanistic insights into the preferential tissue-lineage differentiation phenomenon on the various plastics substrate can be better appreciated from the concept of mechanotransduction – *the process of converting biophysical signals into a series of biochemical intracellular signalling cascades to regulate cellular fate and function* (Tay et al., 2013; Nicolas, 2017a, 2017b). Previous studies have implicated cell shape and cytoskeleton development as important determinants of stem cell differentiation (McBeath et al., 2004; Tay et al., 2015). It has been shown that cell spreading and formation of actin-based stress fiber could initiate a feed-forward loop involving the RhoA-ROCK signalling cascade to increase intracellular actomyosin mediated tension, thereby promoting osteogenesis (McBeath et al., 2004). Consistent with this

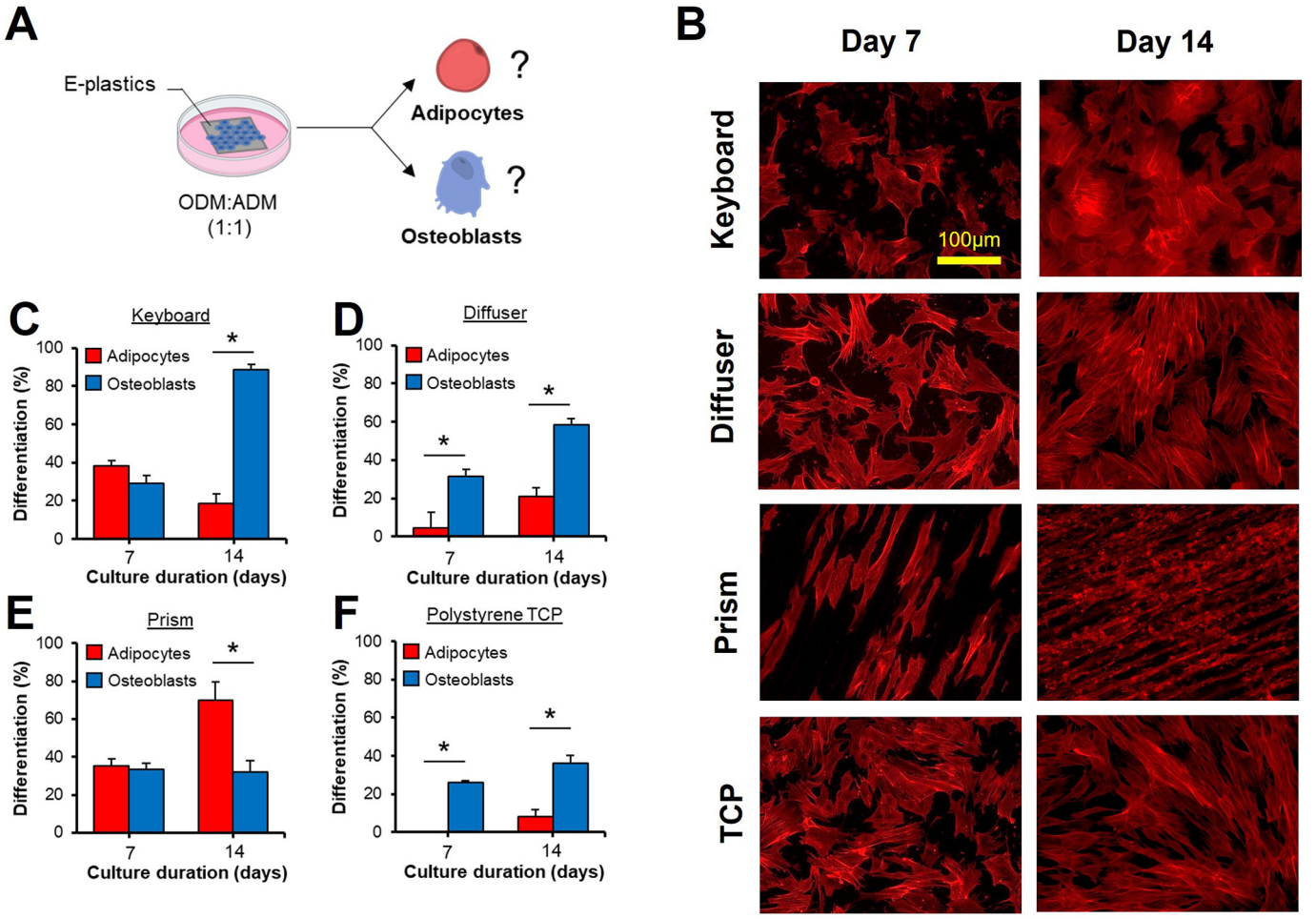


Fig. 5. (A) Schematic illustrating the design of the experiment. ADSCs were cultured on the respective E-plastics consisting of a 1:1 ratio of osteogenic differentiation medium (ODM) and adipogenic differentiation medium (ADM) up to 2 weeks. Cells were then trypsinized and counter-stained for calcium and oil droplets as markers of osteoblasts and adipocytes respectively. (B) Representative fluorescence images of ADSCs on different substrates counter-stained for filamentous actin (red) to unveil the morphology and cytoskeleton development of the cells. Quantitative assessment to determine the percentage of osteoblasts and/or adipocytes on the (C) keyboard, (D) diffuser, (E) prism, and (F) polystyrene TCP substrates. All data are represented as mean ± standard deviation from three experimental replicates. * denotes the statistical difference between the compared experimental groups. Statistical significance is determined at $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

paradigm, ADSCs grown on the keyboard and diffuser E-plastics were well spread, stress fibers were developed and the majority of the cells were directed towards an osteogenic lineage. In contrast, adipocyte differentiation is favoured in MSCs that are characterized with weak cell-material adhesion and under-developed actin cytoskeleton because of limited cell spreading (Liu et al., 2013; Shukla et al., 2016; Khan et al., 2020), as in the case of the cells grown on the prismatic sheet (McBeath et al., 2004).

4. Conclusion

In summary, various E-plastics commonly found in an office setting were systematically characterized to repurpose them for stem cell culture applications. While XRF analysis revealed the presence of several toxic substances, it was found that the leaching of these additives into the cell culture medium was negligible over 2 weeks. Consistent with this observation and earlier report (Shi et al., 2020), In vitro studies showed that these E-plastics can support ADSCs adhesion and proliferation while maintaining its multipotential differentiation properties. The ADSCs displayed different adhesion patterns, morphologies, and cytoskeleton development, that are contingent on the surface roughness, wettability, and topographical features of the E-plastics. Importantly, we demonstrated that E-plastic specific surface properties/features can profoundly influence and direct the differentiation of ADSCs along the osteogenic or adipogenic lineages. From the materials-standpoint, there are, however, two caveats to this study. First, while the characterization of the leches into the cell culture medium is extensive, it is by no means exhaustive. Second, although the ICP-OES data suggest that the leaching of metals into the cell culture medium (37 °C) is insignificant over 2 weeks, it does not exclude the possibility that the leaching of these additives may increase with time. Thus, future studies should focus on the integrated use of other characterization platforms such as high-resolution mass spectrometry to detect and identify other potentially toxic compounds that are uncovered in this study. Furthermore, future studies should also examine in greater detail the long-term outward migration profile of the plastic additives. Going forward, we recommend that there should be greater interdisciplinary efforts to create innovative E-plastics reuse solutions. Concerted attempts by materials scientists, biologists, biomedical engineers, and life science specialists should be directed to (i) ensure the quality of the E-plastics waste; (ii) expand the list of reusable E-plastics that are amenable for basic and advanced cell culture applications; and (iii) better appreciate the long-term effects of E-plastics exposure on the phenotype and performance of stem cells for potential therapeutic gain. Taken together, the findings presented in this study represent a significant paradigm shift in maximizing the value of E-plastics waste and open new "reuse" avenues for end-of-life E-plastics for various in vitro applications.

CRediT authorship contribution statement

Pujiang Shi: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Chiew Kei Tan:** Formal analysis, Investigation. **Zhuoran Wu:** Formal analysis, Investigation. **Jean-Christophe P. Gabriel:** Methodology, Writing – review & editing. **Madhavi Srinivasan:** Writing – review & editing. **Jong-Min Lee:** Writing – review & editing, Funding acquisition. **Chor Yong Tay:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151085.

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