Title: 3D Printed Franz cells – update on optimization of manufacture and evaluation.

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The evaluation of permeation profiles from cosmetic formulations is considered to be a crucial component in both the development and quality assurance of any new product [1, 2]. Data gathered from such studies allow researchers to assess the viability of delivering different materials to and through biological membranes. To date, laboratory *in vitro* permeation processes require the use of modified Franz type diffusion cells, conventionally fabricated from glass, which are available in different formats that can be customised to experimental requirements [3]. We have previously reported on the development of a novel low-cost approach to additive manufactured (AM) Franz diffusion cells and compared the permeation of different active ingredients between glass cells and these newly developed 3D printed constructs. Results suggested a possible interaction of the permeants with the transparent resins used to produce the diffusion apparatus [4]. In the present work, the permeation of caffeine (CAF), a widely studied hydrophilic permeant, was investigated in both AM cells and glass cells [5, 6]. Microscopic analysis of these 3D Franz cells was also conducted in order to assess physical integrity of the scaffolds [7].

Caffeine (CAF) was purchased from Sigma Aldrich, Dorset, UK. Phosphate buffer saline (PBS) tablets were purchased from Oxoid Limited, Basingstoke, UK. Polydimethylsiloxane (PDMS) membrane with a thickness of 250 µm was supplied by Shielding Solutions Limited, Braintree, UK. High performance liquid chromatography (HPLC) grade water and methanol were obtained from Fisher Scientific, Loughborough, UK. Stereolithography resin GPCL04 for printing of transparent materials was purchased from Formlabs, Massachusetts, USA.

AM Franz type diffusion cells were produced using typical glass homologues measurements which were collected using a Vernier callipers (RS Components Ltd., Corby, UK) and drawn *in silico*, using an online computer aided design (CAD) program -

TinkerCAD™ (Autodesk®, California, USA). Graphical designs were then imported to the Preform™ software tool (version: 2.14.0, Formlabs, Massachusetts, USA) prior to printing. Stereolithography (SLA) 3D printing was carried out using a Form2 printer (Formlabs, Massachusetts, USA). The 3D printed receptor compartment was reproduced with the following specifications: outer diameter (O.D.) = 30 mm, inner diameter (I.D.) = 10 mm, height (*h*) = 16 mm and aliquot collection arm (length (*l*) = 54 mm with I.D. = 3 mm) as shown in Fig. 1. The inner object volume was 2.33 ± 0.03 cm^3 (weight (*w*) = 499.23 ± 0.41 mg) for the receptor compartment of the AM Franz cell. The donor compartment was printed with O.D. = 30 mm, I.D. = 10 mm, *h* = 10.7 mm (Fig. 1) which gave a total inner object volume of 1.15 ± 0.01 cm^3 (*w* = 309.22 ± 0.30 mg). Printing was carried out using 100 μm resolution and a resin tank temperature of 28 ºC. Preform™ generated supports (point size: 0.50 mm, point density: 1.0) were used for all printed scaffolds. Post-curing of AM constructs was achieved by exposing the 3D printed Franz type cell compartments to UV light (405 nm) at 60 °C for 15 min using Form Cure (Formlabs, Massachusetts, USA) [8]. All transparent 3D printed Franz cells were tested for leaks by filling both compartments with a PBS (pH 7.3 ± 0.1) solution. These printouts (compartments) were clamped together using in-house manufactured metallic clamps. The AM cells were examined for leaks over a minimum of 24 h and the printouts were considered successful if no aqueous media was present on the outer wall after this period.

Caffeine analysis was conducted using a HPLC (Agilent Technologies 1200 series) equipped with an Agilent G1322A degasser, G1311A quaternary pump, G1329A auto sampler and G1316A thermostat column compartment (Agilent Technologies, Cheadle, UK). The analysis was performed using a Phenomenex Luna Phenyl Hexyl column fitted with a guard column (Phenomenex, Cheshire, UK). The length, internal diameter and particle size were 250 mm, 4.6 mm and 5 μm, respectively. The mobile phase consisted of water:methanol (40:60). Prior to its use, the mobile phase was degassed using an ultrasonicator (VWR International, Lutterworth, UK) to remove air bubbles. The flow rate of the mobile phase was 1 mL min^{-1} and the column temperature was set at 23 °C. The chromatogram was acquired at a wavelength of 278 nm. A sample volume of 10 μL was injected for a total run time of 10 min. A known amount of CAF was dissolved in PBS (pH 7.3 ± 0.1) and a stock solution (1000 μg mL^{-1}) was prepared. The stock solution was diluted to prepare various concentrations of CAF. The CAF peak was evident at 3.9 min. The calibration curve was constructed in the concentration range of 0.05 – 500 μg mL^{-1}. A linear relationship was found

between concentration and peak area with regression coefficient values (r^2) of greater than 0.999. The LOQ and LOD values were 0.5 and 0.05 μg mL^{-1}, respectively [9].

Caffeine compatibility studies with AM Franz type diffusion cells were conducted by filling the diffusion cell receptor compartment with a 1.5 mg mL^{-1} CAF solution prepared in PBS (pH 7.3 ± 0.1). The AM Franz cell was then sealed with Parafilm® (Bemis NA, Neenah, USA) and placed in a JB Nova thermostatically controlled water bath (Grant, London, UK) set to 32 ± 1 °C equipped with a HP 15 stirring system (Variomag, Florida, USA). 200 μL aliquots were taken from the Franz cell receptor compartment at different timepoints, 0, 24, 48 and 72 h and replaced with the same volume of fresh CAF solution. The samples were appropriately diluted to be in the range of the calibration curve and analysed using HPLC.

The surface morphology of the AM Franz type diffusion cells from pre and post-compatibility studies was analysed using an EVO MA 10 (Carl Zeiss SMT GmbH, Oberkochen, Germany) scanning electron microscope (SEM). Constructs were cut in different cross-sectional directions and sputter coated with gold for 45 s using an Emitech 550 (Emitech Ltd., Ashford, UK). The 3D printed materials were then attached onto adhesive carbon disks (Fig. 2) and SEM micrographs were taken of different longitudinal and cross-sections. SEM was conducted at low magnification, with an electron accelerating voltage of 10 – 20 kV under vacuum. Images of the surface structure of the samples were captured and collected using the Everhart-Thornley detector image acquisition and processing software (Carl Zeiss SMT GmbH, Oberkochen, Germany).

In vitro permeation studies in both glass and AM Franz type diffusion cells were conducted using 1 mL CAF (1.5 mg mL^{-1}) solution, applied to a PDMS model membrane. Freshly prepared PBS (pH 7.3 ± 0.1) was used as the receptor solution. The temperature of the PDMS membrane was equilibrated to 32 ± 1 °C and the CAF solution applied. The donor compartment was not occluded after application of the CAF solution. Samples of 200 μL of receptor solution were removed from the receptor compartment at various time intervals (0, 5, 10, 15, 30, 45, 60, 90, 120 and 180 min), and replaced with fresh temperature equilibrated PBS solution. The samples were appropriately diluted to be in the range of the calibration curve and analysed using HPLC.

Glass diffusion cells are conventionally used in dermal permeation studies given their lack of interaction with permeants [10]. However, high-costs are associated with this type of glass apparatus and they are not mechanically robust. We previously reported the development of 3D printed Franz diffusion cells in different permeation experiments with results showing chemical incompatibility between the electron rich domains of permeants and

the chemical structure of the polymer in the printed constructs [4]. In this study, CAF was chosen as a model molecular permeant because of its hydrophilic nature (Table I) and wide use in skin permeation experiments [6, 9, 11, 12]. With a log $P_{(o/w)}$ value of -0.07 and an aqueous solubility of 21.6 mg mL^{-1}, CAF was initially screened for its AM Franz diffusion cell compatibility prior to conducting *in vitro* permeation studies. Stability testing of CAF with the Formlabs clear resin GPCL04 confirmed comparable outcomes as for glass diffusion cells, with results demonstrating similar recovery between the two constructs (1.49 ± 0.01 and 1.50 ± 0.01 mg mL^{-1}, respectively) after 72 h as reported in Fig. 3 (Two way ANOVA Tukey´s multiple comparisons). This suggested that no interaction occurred between CAF and the transparent 3D printed scaffold indicating the suitability of these AM constructs to be used for permeation studies.

The morphology of the AM Franz type diffusion cells surfaces was characterised using SEM. As shown in Fig. 4, different cross-sectional images of the 3D printed constructs using 54 to 256 × magnifications confirmed the structural similarity between AM scaffolds studied prior (Fig. 4 A-C) and post-compatibility (Fig. 4 D-F). No significant differences were visible from the SEM micrographs demonstrating consistent, smooth and non-porous surfaces of the 3D printed Franz cells' core structure. Additionally, shape and size uniformity across all printed samples was consistent for both compatibility and permeation studies with no evident swelling of the polymeric constructs.

In vitro permeation studies showed that similar permeation of CAF was observed in AM diffusion cells when compared with the Franz type glass cells as shown in Fig. 5. All experiments were conducted using the same type of PDMS membrane, confirming the suitability of CAF as a model molecular permeant with the GPCL04 AM resin. Permeation studies using transparent 3D printed constructs resulted in 12.85 ± 0.53 µg cm^{-2} CAF recovery in the receptor solution after 180 min with a comparable CAF permeation, 11.49 ± 1.04 µg cm^{-2}, for the glass homologues.

Similar to their glass counterparts, 3D printed Franz type diffusion cells were shown to be structurally solid with SEM analysis displaying no printout degradation during the AM resin/model active compatibility studies. Also, subsequent comparative permeation analysis conducted in typical glass diffusion cells and 3D printed scaffolds using CAF showed consistent results. Physical and/or chemical interactions between printing resins and other model active ingredients was previously described as a limitation for the use of AM technology for Franz cell fabrication [4]. Here we have demonstrated the relationship of using a more hydrophilic molecular entity as an alternative for the testing of these 3D printed

transparent constructs. This choice relied on our previously reported methodology that showed improved compatibility between the polymeric scaffold and the model tested compounds when less lipophilic permeants were used (i.e. niacinamide, terbinafine hydrochloride and diclofenac free acid) [4]. Hence, the increase in compound recovery translates into a robust analytical process suitable to be used in real-life training scenarios. With a price range of 2 – 3 USD per set (i.e. Franz cell receptor and donor compartments) and a printing time of ~3.5 hours to reproduce four full sets, AM technology may now be considered as a viable alternative to the use of conventional glass cells.

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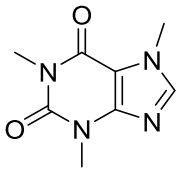
Table I – Physicochemical properties of caffeine (CAF)

Chemical structure	
Molecular weight (Da)	194.19
log P (*o/w*)	-0.07 [13]
Aqueous solubility (mg mL^{-1}, 25 °C, pH 7.0)	21.6 [14]

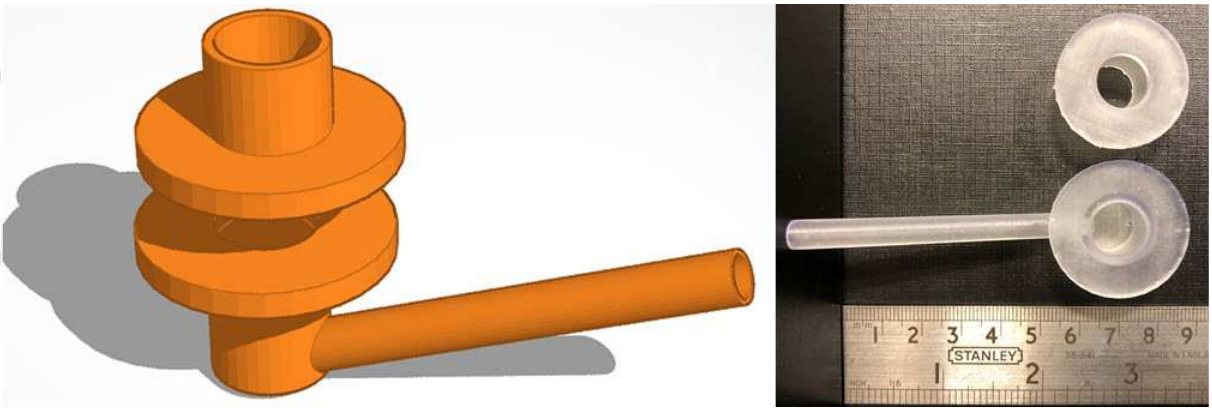


Figure 1 – AM Franz diffusion type cell design generated in TinkerCAD™ (*left*) and cell printout top view (*right*).

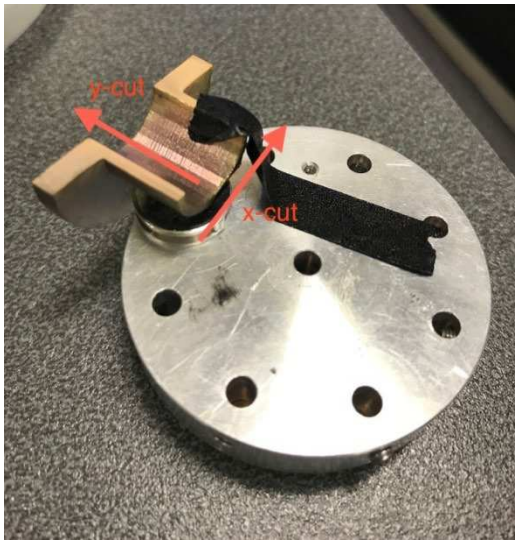


Figure 2 – Sputter coated AM Franz type diffusion cell attached onto adhesive carbon disk before SEM imaging with *x* and *y* axis cut edges.

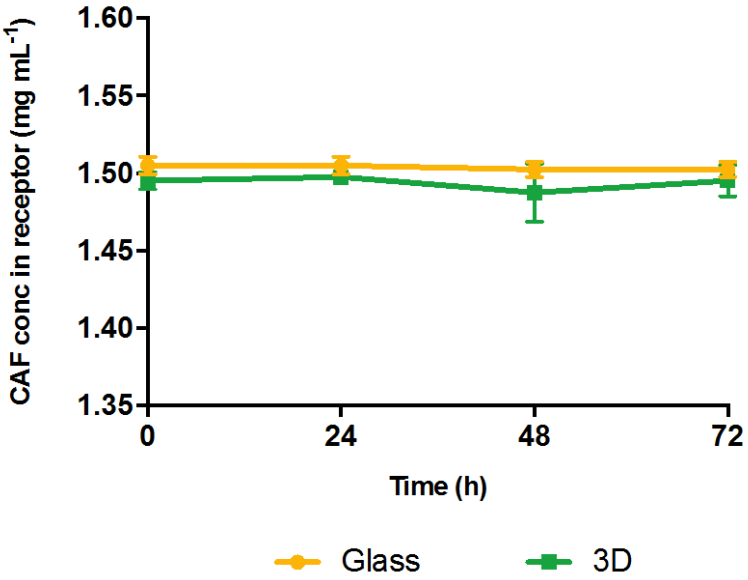


Figure 3 – Amount in mg mL^{-1} of CAF recovery from glass and 3D printed receptor compartments at 0, 24, 48 and 72 h (n=4, mean ± SD).

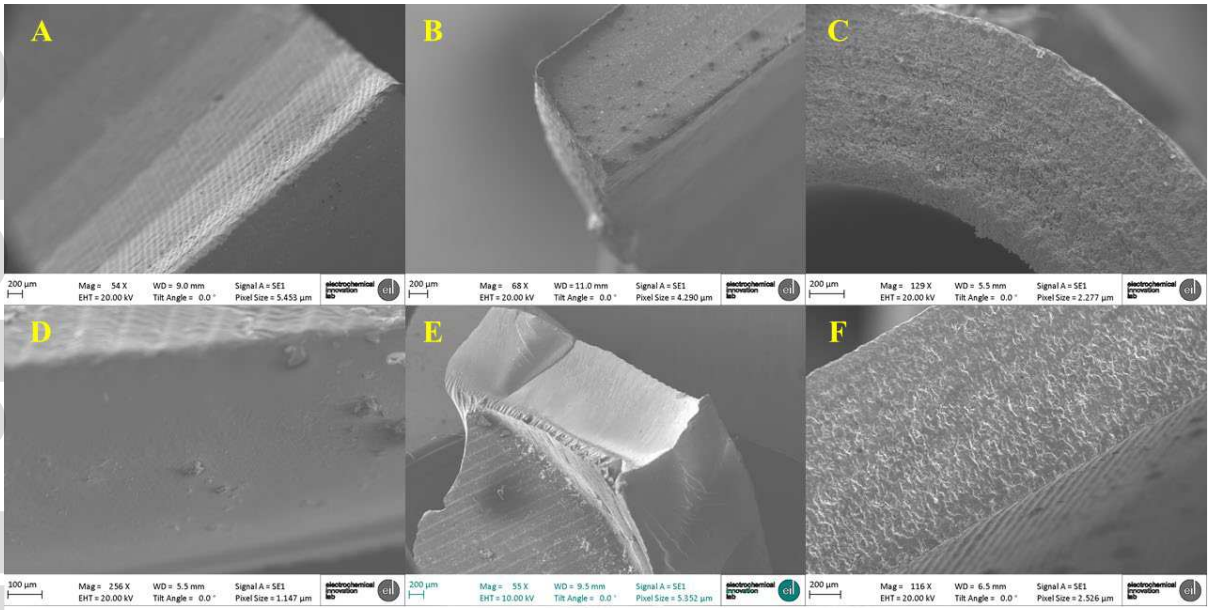


Figure 4 – SEM micrographs of AM Franz type diffusion cell from pre-compatibility study: (A) inner wall receptor compartment, (B) y-edge overview receptor compartment, (C) full section collection arm; and after 72 h post-compatibility study: (D) cut inner wall receptor compartment, (E) overview wall receptor compartment, (F) overview of edge cut from collection arm.

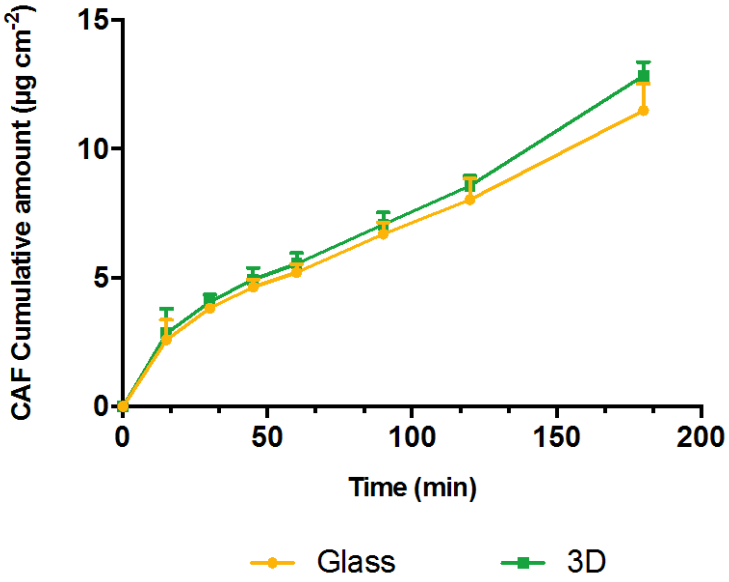


Figure 5 – Amount of CAF permeation from initial dose per cm^2 of PDMS membrane at 0, 15, 30, 45, 60, 90, 120 and 180 min using glass and 3D printed Franz type diffusion cells (n=4, mean ± SD).