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Title: 3D-printed Franz type diffusion cells.

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Introduction

Diffusion cells are routinely used for the study and analysis of permeation of active compounds through biological and synthetic membranes. The data from such studies are important in determining the feasibility of delivering materials to and through the skin [1]. Conventional cells are typically fabricated from glass and are available in a range of shapes, sizes and may be modified depending on the required experimental conditions [2]. As a consequence, these cells are fragile and require careful handling to ensure that they withstand the robust procedures required for performance of skin penetration and mass balance studies.

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Emerging enabling technologies, such as 3D printing, permit the facile additive manufacturing of parts required in research laboratories using different polymeric materials. These have shown excellent potential for the production of diverse pieces of laboratory equipment [3]. The relative ease of design combined with comparatively low-cost materials have led to 3D printers becoming essential apparatus in any research laboratory [3, 4].

The aim of the present work was to develop a robust method for construction of Franz-type diffusion cells using 3D printing. This process should provide a substantial reduction in production costs as well as time required for manufacture. The important parameters for a 3D printing study include object design, choice of printing resin, printout curing/post-curing of resin settings and necessity for additional resin coatings. Moreover, this methodology will allow precise control of all processing variables and as such, should also improve the accuracy and reproducibility of Franz diffusion cell design. The physical properties and permeant compatibility of these novel 3D printed platforms was evaluated with a range of model active compounds and aqueous media conventionally used in permeation studies. Although a number of resins are commercially available for 3D printing, to our knowledge no attempts have been made to use them to fabricate transparent Franz diffusion cells. Visual inspection of the receptor phase of Franz cells is necessary when conducting permeation studies to ensure the absence of air bubbles and to confirm that the cell assembly is leak-proof. Typically, the types of resins available are defined as standard, rigid, tough, durable, flexible and temperature resistant. These resins are also offered by manufacturers in different ranges of colours, suitable for a vast variety of applications. As a starting point the acrylate-based clear resin GPCL04 supplied by Formlabs was chosen since it has been used successfully for the manufacture of equipment parts that must be transparent or necessitate the visualisation of internal features.

Materials and Methods

Materials

Terbinafine hydrochloride (TBF) was obtained from Shandong Qihe Yinfeida Chemical Co. Ltd, Jinan, China; niacinamide (NIA), diclofenac free acid (DFA), n-methyl paraben (MPB), phosphate buffer saline (PBS) tablets and 6% w/v polyoxyethylene (20) oleyl ether were all purchased from Sigma Aldrich, Dorset, 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, methanol and acetonitrile were obtained from Fisher Scientific, Loughborough, UK. Stereolithography resins suitable for printing transparent materials were either purchased from Formlabs (resin code: GPCL04, Formlabs, Massachusetts, USA) or formulated in-house as reported previously [5]; these light-curable resins are acrylate based with details of the resin

composition and synthetic routes of development disclosed elsewhere. The plastic coating materials used were Sigmacote (Sigma Aldrich, Dorset, UK), selectophore (Sigma Aldrich, Dorset, UK), SKU 660-500-de (ALGT, Velen, Germany) and SiO_2 plastic protect (SiO_2, Toronto, Canada).

Development of 3D printed Franz diffusion cell prototype

Glass Franz diffusion cells were measured using Vernier callipers (RS Components Ltd., Corby, UK) and drawn *in silico*, using an online computer aided design (CAD) program - TinkerCAD™ (Autodesk®, California, USA). Graphical images of the designs were imported to the Preform™ software tool (version: 2.14.0, Formlabs, Massachusetts, USA) prior to printing. 3D printing was carried out using two different stereolithography (SLA) machines: Form1+ and Form2 (Formlabs, Massachusetts, USA).

The 3D printed receptor compartment was printed 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. This resulted in an inner object volume of 2.32 ± 0.03 cm^3 (weight (*w*)= 496.05 ± 0.44 mg) for the receptor compartment of the 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 1.12 ± 0.01 cm^3 (*w* = 309.71± 0.31 mg). Printing was carried out using a resolution of 100 μm per resin layer and a resin tank temperature of 28 ºC. In producing 3D printed materials, supports are required for removal of the manufactured object from the building platform at the end of the process; Preform™ generated supports (point size: 0.50 mm, point density: 1) were selected in this study. Post-curing of resins was achieved by exposing the 3D printed Franz cell compartments to UV light (405 nm) at 60 °C for 15min using the Form Cure equipment (Formlabs, Massachusetts, USA) [6]. All transparent 3D printed Franz cells were tested for leaks by filling both compartments with an aqueous phosphate buffer saline solution. These printouts (compartments) were clamped together using in-house manufactured metallic clamps. The 3D printed set-up was examined for leaks over a minimum of 24 hours and the printouts were considered successful if no aqueous media was present on the outer wall after this period.

Compatibility study of model actives and 3D printed Franz diffusion cells

Four solutions of the model actives were prepared as follows: 250 μg mL^{-1} n-methyl paraben (MPB), 50 μg mL^{-1} niacinamide (NIA), 50 μg mL^{-1} terbinafine hydrochloride (TBF) and 50 μg mL^{-1} diclofenac free acid (DFA). These concentrations were selected based on previous permeation studies with the active compounds in conventional Franz cells. MPB and NIA were prepared in PBS solution

(pH 7.3 ± 0.1). TBF and DFA were prepared in a 6% w/v polyoxyethylene (20) oleyl ether aqueous solution, the non-ionic surfactant solution chosen to increase chemical solubility of the compounds.

The 3D printed Franz diffusion cell receptor compartment was filled with the model active solution, 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 solution of the respective active compound. Samples were appropriately diluted to be in the range of the relevant calibration curves and analysed using HPLC. The HPLC methods have previously been reported and/or validated according to ICH guidelines [7-11].

In vitro permeation studies of MPB using glass and 3D printed Franz diffusion cells with PDMS membrane

Permeation studies in both glass and 3D printed Franz diffusion cells were conducted using 50 µL MPB (1.5 mg mL^{-1}), applied to a PDMS model membrane. Freshly prepared PBS (pH 7.3 ± 0.1) was used as the receptor solution in all studies. The temperature of the PDMS membrane was equilibrated to 32 ± 1 °C and the solution applied using an Eppendorf Multipette. The donor compartment was occluded using Parafilm® after application of the MPB solution. Samples of 200 µL of receptor solution were removed from the receptor compartment at various time intervals (0, 15, 30, 45, 60, 90, 120, 150 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 following previously validated methods according to ICH guidelines [10].

Coating of 3D printed Franz cells and compatibility study with MPB

Coatings were applied to the 3D printed Franz diffusion cells as per manufacturers' recommendations: an excess of the coating was applied to the cell surfaces and left to rest for a minimum period of 30 min; excess coating was then removed. Table I details the specific coating and post-coating curing times for each material. The receptor compartment of the coated Franz diffusion cells was filled with MPB solution (1.5 mg mL^{-1}), sealed with Parafilm® and placed in a JB Nova thermostatically controlled water bath set to 32 ± 1 °C equipped with a HP 15 stirring system. 200 µL aliquots were taken from the Franz cell receptor compartment at different time points: 0, 24, 48 and 72 h and replaced with 200 µL MPB solution (1.5 mg mL^{-1}). The samples were appropriately diluted

and analysed using HPLC, following previously validated methods according to ICH guidelines [7, 10].

Results and Discussion

Compatibility study of model actives and 3D printed Franz diffusion cells

Glass diffusion cells are traditionally used in dermal penetration studies given their lack of interaction with active ingredients [12]. Stability studies, previously conducted with conventional Franz diffusion cells by our group, have also confirmed that glass is inert to these active compounds. Prior to conducting *in vitro* permeation studies, the 3D printed Franz cell receptor compartment was initially screened for compatibility with solutions of the model actives selected (i.e. NIA, TBF, DFA, MPB). Compared with the glass cells, a decrease in recovery of all compounds in the receptor compartment was evident when using transparent 3D printed Franz diffusion cells as shown in Fig. 2. The decline in concentration of active compounds was particularly significant for MPB (46.2 ± 13.1%) when compared with NIA (25.1 ± 4.0%), TBF (18.5 ± 12.0%) and DFA (9.8 ± 12.9%) from its initial starting concentrations recovered after 72 h (ANOVA, $p<0.05$). This suggested an interaction between the 3D printed polymeric resin and MPB. As a result of this significant decrease in recovered material, subsequent compatibility investigations of the resins were conducted only using MPB as the model active ingredient.

Formlabs clear resin GPCL04 is primarily composed of methacrylated oligomers and monomers (i.e. acrylic components having one or more epoxy, vinyl ether, vinylcaprolactam and vinylpyrrolidone substituents). According to manufacturer's literature these materials are comparatively hydrophobic [13, 14]. As shown in Table II, the molecules chosen for the preliminary compatibility experiments have a range of physicochemical properties. These model active ingredients, that have a diverse range of molecular weights, ranging from 122.12 Da (NIA) to 296.15 Da (DFA) also vary in solubility and log $P_{(o/w)}$ values. With a log $P_{(o/w)}$ value of 1.96 and a molecular weight of 152.15 Da, MPB showed the greatest interaction and subsequent decrease in concentration when exposed to the 3D printed cells throughout the experiments. This may reflect a possible chemical interaction between the electron rich domains of MPB and the methacrylated groups of the resin. Although there is also a possibility of some uncured resin interacting with MPB this appears to be unlikely given that the procedures adhered to manufacturer's protocols [6]. Furthermore, previous studies conducted by Zaleski and co-workers have shown that interactions between low molecular size permeants are influenced by the porosity of polymeric materials [15]. This will be further investigated in a future study using electron microscopy.

In vitro permeation studies of MPB using glass and 3D printed Franz diffusion cells with PDMS membrane

Statistically lower permeation of MPB was observed in the 3D printed Franz cells compared with conventional glass cells as shown in Fig. 3 (ANOVA, $p<0.05$). All experiments were conducted using the same PDMS membrane, again demonstrating that the results indicate a direct interaction of MPB with the 3D diffusion cell resin. Permeation studies using glass cells resulted in 77.8 ± 2.9% MPB recovery of the initial dose applied in the receptor solution after 72 h. A decrease of 51.4 ± 3.7% (Formlabs resin) and 94.4 ± 3.5% (in-house resin) in MPB recovery was seen for the two transparent resins evaluated printouts when compared to its glass alike after the same 72 h permeation period (Fig. 3).

Coating of 3D printed Franz cells and compatibility study with MPB

In order to address, and limit, the physical and/or chemical interactions between the 3D printed Franz diffusion cell resin and MPB, a range of commercially available plastic coatings were applied to the 3D printed Franz cells (Table I). These coatings were selected as they are commonly used in different industries as part of the manufacturing process of plastics or as a refinement of the finished products. With appropriate adhesive and chemical properties that enable the creation of hydrophobic environments, these coatings would potentially increase MPB recovery. As shown in Fig. 4, the concentration of MPB continued to decrease on exposure to different types of coatings over 72 h. This could be the result of the possible above-mentioned interaction between MPB and the polymeric 3D printed structure, but also with the coating materials used for each printout, further reducing the compounds' recovery. The conditions for coating and post-coating applications (Table I) were also varied. By increasing the time of exposure and introduction of a new post-curing cycle, our rationale was to optimise adhesiveness of the coating to the transparent 3D printed Franz cell surfaces. However, no improvements in the recovery of MPB after incubation studies were evident (data not shown here).

Conclusion

In this proof of concept study, 3D printing was used to fabricate Franz diffusion cells. These newly developed printouts were proven architecturally robust since no leaks, cracks or polymeric degradation were found throughout all experiments. Future studies will be conducted in order to demonstrate 3D printed diffusion cells re-usability as per its glass counterparts. 3D printing has shown to be an effective, rapid and cost-effective process for the manufacture of laboratory apparatus. This concept was supported in our studies by the time needed to print three sets of Franz diffusion

cells (3 hours) with a total cost of 3 USD per set (i.e. receptor and donor compartments). Permeation of active ingredients was also compared between glass and transparent 3D printed diffusion cells. Subsequently the cells were evaluated with a range of active ingredients. The findings suggest a physical and/or chemical interaction with the resins used to produce the cells. However, the resins currently available for production of laboratory apparatus that should be transparent, i.e. Franz cells, are limited. Other resins are currently in development given the relatively recent advent of 3D printing technologies and these will be explored in a future study. The use of scanning electron microscopy (SEM) in our forthcoming studies will allow for the matrix of the transparent 3D cell surfaces to be surveyed and should allow further insight into methods to limit the active-polymer interactions observed in the present work.

Finally, the hydrophobic coatings used in these studies did not improve recovery of the active ingredients studied despite their widespread use as barrier materials for other basic laboratory apparatus. Therefore, further testing of additional commercial and non-commercial coatings will also be pursued. Any new cell design and its construction materials must also be tested for compatibility with a range of permeants.

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Table I – Specifications for Franz diffusion cells curing and post-curing times (405 nm light exposure at 60 °C)

Franz cell (material)	**Post-curing time (minutes)**	**Coating**	**Coating time (hours)**	**Post-coating curing time (minutes)**
Glass	-	-	-	-
Formlabs clear resin GPCL04	15	-	-	-
		Sigmacote	6	30
			12	30
			6	45
			12	45
		Selectophore	12	30
		SKU 660-500-de	12	30
		SiO_2 plastic protect	12	30
In-house resin	15	-	-	-

Table II – Physicochemical properties of active ingredients and preservatives used in the studies

Compound	Molecular weight (Da)	log P (*o/w*)	Aqueous solubility (mg mL^{-1}, 25 °C, pH 7.0)
Niacinamide (NIA)	122.12	-0.37 [16]	500 [17]
Terbinafine hydrochloride (TBF)	291.43	5.90 [18]	0.74 [19]
Diclofenac free acid (DFA)	296.15	4.51 [20]	0.00237 [21]
n-Methylparaben (MPB)	152.15	1.96 [16]	2.50 [22]