Fused deposition modelling for the development of antiplatelet materials for cardiovascular applications

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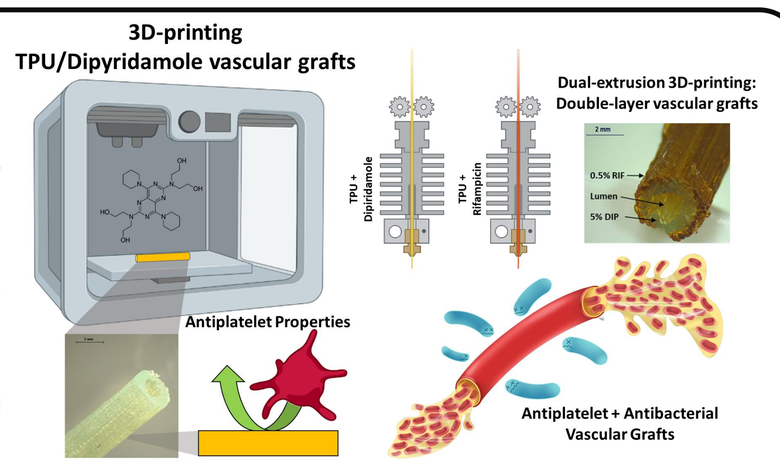
Abstract

This work describes the use of fused deposition modelling (FDM) to prepare antiplatelet thermoplastic polyurethane (TPU)-based small diameter vascular grafts (SDVGs). FDM 3D printing technology is widely available and provides the ability to easily design SDVGs on demand, enabling to customize the dimensions of the vascular prosthesis. An antiplatelet drug, dipyridamole (DIP), was combined with TPU using hot-melt extrusion to prepare filaments. DIP cargos ranged between 5 and 20% (w/w). The resulting filaments were used to prepare SDVGs using FDM. These grafts were physicochemically characterised and their performance was evaluated by testing DIP release kinetics, antiplatelet activity and the *in vitro* hemocompatibility. The results suggested these grafts were capable of providing a sustained DIP release for 30 days. Moreover, the resulting grafts loaded with 5% DIP showed a clear antiplatelet effect as opposed to grafts containing higher DIP cargos. The latter was related to sample surface roughness and hydrophilicity/hydrophobicity, as well as the drug loadings. Finally, DIP loaded TPU was combined with rifampicin (RIF)-loaded TPU to prepare double-layered SDVGs. These grafts showed a clear antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

Experimental workflow

- The integration of DIP within the TPU matrix (pre-extrusion process) was performed by using a plastograph Brabender, what enabled the adding of high DIP percentages (up to 20%) while maintaining the homogeneity of the resulting material.
- The obtained filaments were then used to 3D print SDVGs containing different DIP percentages by using FDM 3D printing technology (Ultimaker 3)
- A TPU filament containing 0.5% RIF was used in the second extruder to prepare a double-layered SDVG from the same 3D printer.
- The produced SDVGs were fully characterized through multiple techniques such as scanning electron microscopy (SEM), contact angle goniometry (CAG), Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) analysis and thermal analysis: thermogravimetric (TGA) and differential scanning calorimetry (DSC) analysis.
- The drug release, antiplatelet effect and *in vitro* hemocompatibility of the SDVGs, as well as the antimicrobial activity of the double-layered SDVGs against *Staphylococcus aureus* NCTC 10788 (Gram-positive) and *Escherichia coli* NCTC 10418 (Gram-negative) were also evaluated.

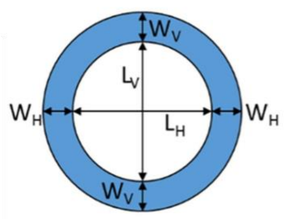


Results

1. Preparation of DIP-eluting SDVGs

A

3D printed model distance (mm)				
	L_V	L_H	W_V	W_H
CAD Model	2	2	0.5	0.5
TPU	2.13 ± 0.13	1.69 ± 0.08	0.50 ± 0.08	0.59 ± 0.05
5% DIP	1.90 ± 0.07	1.67 ± 0.14	0.52 ± 0.10	0.77 ± 0.06
10% DIP	1.45 ± 0.11	1.55 ± 0.07	0.65 ± 0.20	0.92 ± 0.13
20% DIP	0.95 ± 0.15	1.20 ± 0.10	0.88 ± 0.29	1.23 ± 0.08



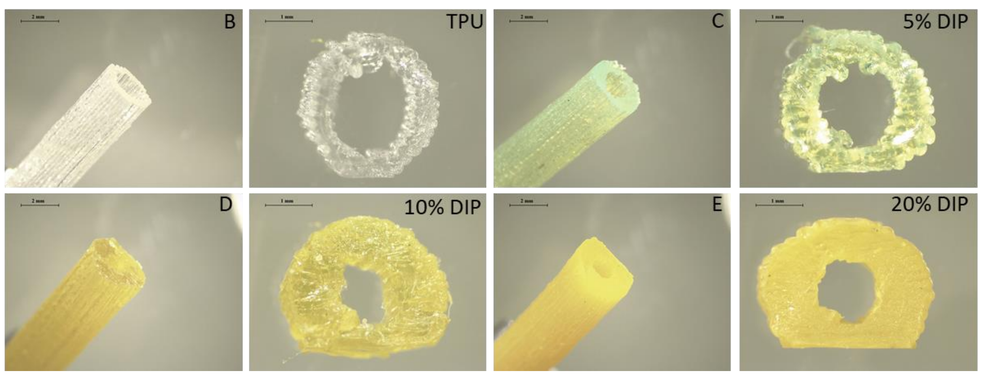


Figure 1: Table showing the dimension of the 3D-printed SDVGs and the cross-section diagram of them with legend for these dimensions (n ≥ 4) (A). Light microscope images of the 3D-printed SDVGs and their cross-sections (B-E). SDVGs containing 5% DIP presented small differences when compared with the original computer-aided design (CAD) file

2. SEM images of DIP-eluting SDVGs

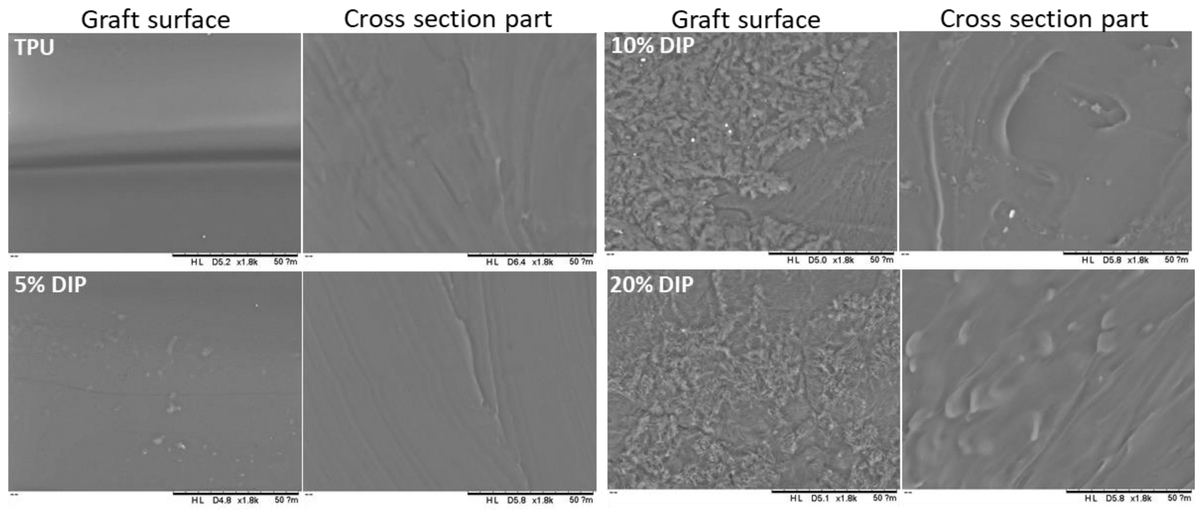


Figure 2: SEM images of the 3D-printed SDVGs surfaces containing different DIP concentrations and their cross sections. The SEM images showed that 3D-printed SDVGs containing no DIP and 5% DIP presented smoother surfaces in comparison with the SDVGs prepared with higher concentrations of DIP, which is ultimately influencing on the platelet adhesion.

3. Physicochemical characterisation DIP-eluting SDVGs

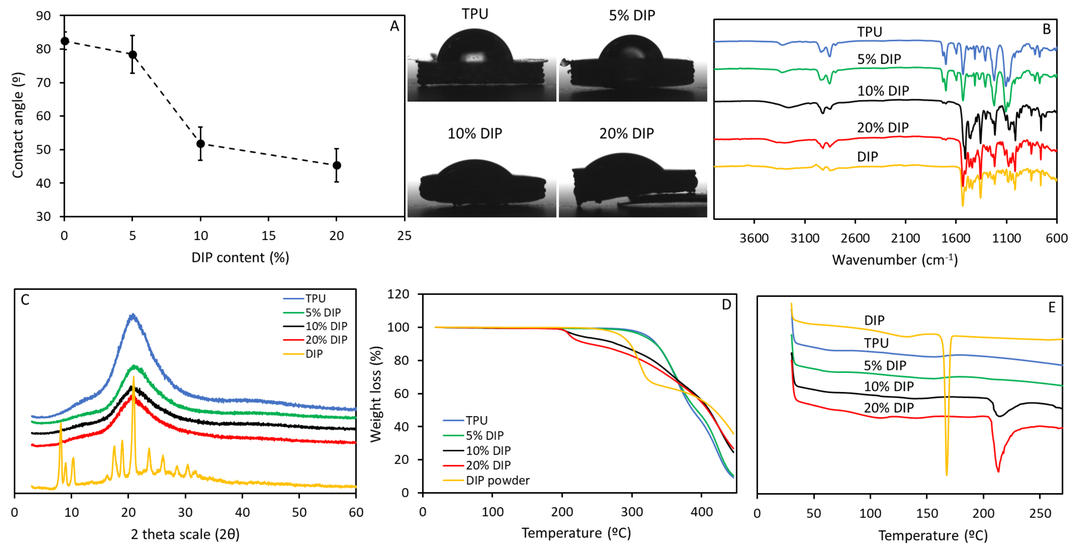


Figure 3: Influence of the DIP content on the contact angle of water with the DIP-loaded 3D-printed samples (n = 4) (A). FTIR spectra (B), XRD diffractograms (C) and TGA (D) and DSC curves (E) of each of the DIP-loaded 3D-printed samples. The contact angle measurements reported an increase in the surface hydrophilicity of the 3D printed scaffolds after increasing the DIP loadings (10% and 20%).

4. *In vitro* drug release

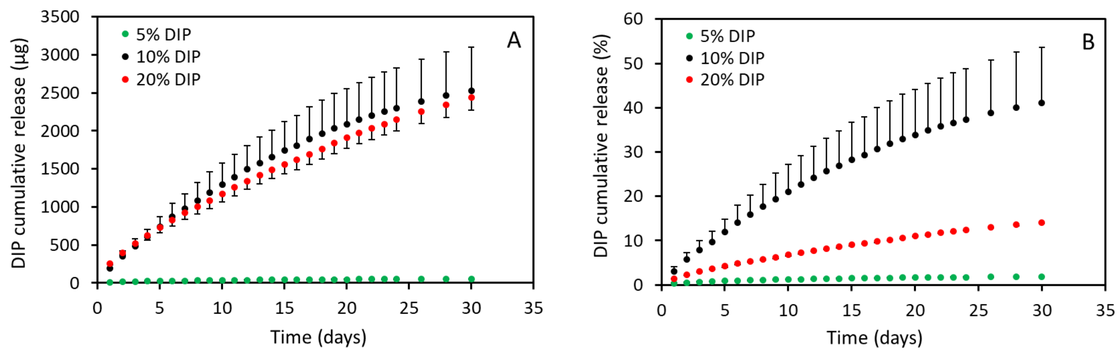


Figure 4: I*n vitro* DIP release curves from DIP-eluting 3D-printed SDVGs up to 30 days in PBS at 37°C expressed in µg as function of time (A) and expressed in percentage as a function of initial DIP drug loading (B) (n = 4). DIP-eluting SDVGs showed a sustained drug release for 30 days and no obvious burst release was observed within the first 24 h.

5. Platelet adhesion and hemocompatibility of SDVGs

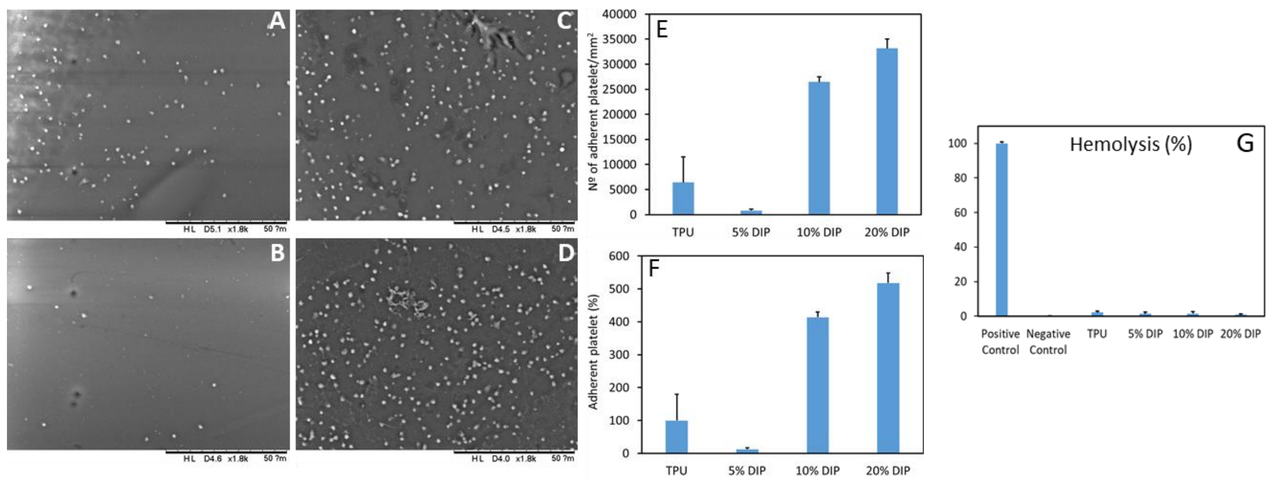


Figure 5: SEM images of rabbit blood platelet depositions on the surfaces of TPU (A), 5% DIP (B), 10% DIP (C) and 20% DIP-loaded 3D-printed samples (D). Results of the platelet adhesion study expressed in platelet/mm^2 (E), and expressed in percentage of platelets adhered to the samples surface, using TPU as reference (F). Rabbit blood hemolysis percentages of the DIP-loaded 3D-printed samples (G) (n = 5). This experiment showed that 5% DIP-loaded samples presented a clear antiplatelet effect. These results, therefore, highlights the importance of the DIP loading into the material.

6. Antimicrobial properties of the double-layered SDVGs

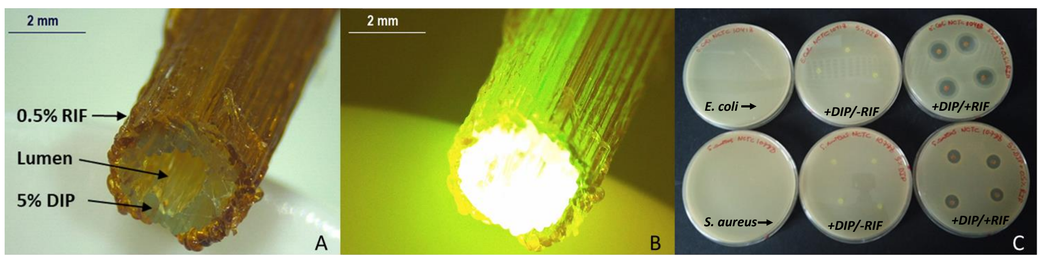


Figure 6: Light microscope image of a double-layered SDVG (A) and the same image using the NIGHTSEA Model SFA Stereomicroscope Fluorescence Adapter (B). A picture showing the zones of inhibition obtained for *S. aureus* and *E. coli* in MH agar using slices of the double-layered SDVG (C).

Conclusions

- The characterisation of the resulting SDVGs suggested that after the extrusion and printing process the drug present in the samples was in an amorphous state. Moreover, 3D-printed SDVGs using filaments loaded with up to 5% (DIP) presented small differences when compared with the original CAD file.
- The results suggested that samples loaded with 5% DIP presented the highest antiplatelet activity. This can be due to its smoother and more hydrophobic surface. Therefore, these results are confirming the influence on the platelet adhesion of the surface roughness and hydrophilicity of the performed 3D-printed materials. Furthermore, all the 3D-printed samples prepared in this work were hemocompatible.
- A double-layered SDVG containing two different drugs DIP and RIF was also prepared and exhibited a clear antimicrobial activity against *S. aureus* and *E. coli*, pathogens involved in hospital-acquired infections (HAI)