

## Surface properties and biocompatibility of solvent-cast poly[ $\epsilon$ -caprolactone] films

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### Abstract

Poly( $\epsilon$ -caprolactone) (PCL) was dissolved in four solvent systems, chloroform, tetrahydrofuran, acetone and ethyl acetate, and cast onto glass Petri dishes. The surface properties of the resulting films were investigated. The extent to which their properties were determined by the solvent used in each case was quantified in terms of contact angle, surface morphology, attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), and the adhesion and proliferation of fibroblasts by direct contact. The surface of the PCL film in contact with glass was denoted the SG surface, and the other, which was exposed to the gas phase, a mixture of air and residual solvent vapour, was denoted the SA surface. In the case of hydrophobic solvent systems, the advancing contact angle of the SG surface was always lower than that of the SA surface. With hydrophilic solvent systems, on the other hand, the advancing contact angle of the SG film surface was higher when the contact angle of the Petri dish was higher than that of the gaseous mixture of the air and solvent vapour, otherwise it was lower or equal to that of the surface on which it was cast. The surface morphology was dictated by the solubility of PCL in the respective solvent systems: high dissolution solvents such as chloroform and tetrahydrofuran produced films that comprised PCL aggregates, the particles being larger in the case of chloroform, whereas the less efficient solvents (acetone and ethyl acetate) resulted in a filamentous structure. The ATR-FTIR results confirmed that the chemistry of the SA surfaces differed according to the solvent system used. Preliminary cell culture experiments carried out with the PCL films established that murine (L929) fibroblasts grew well on all surfaces regardless of the solvent used, although the rates of adhesion and proliferation were not as great as on tissue culture plastic controls. Of all the surfaces examined in this study, the cells favoured the SG aspect of ethyl acetate cast PCL films, the surface of which had the finest pore size and relatively low contact angle.

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### 1. Introduction

The goal of Tissue Engineering research is to reconstitute living cells and other natural substances in the form of tissue substitutes that can be used to repair, maintain or enhance normal bodily structure and function. Such tissue regeneration is often achieved with the aid of biodegradable polymers, onto which cells may be seeded, and which are introduced into a patient where the material gradually resorbs, leaving behind a matrix of connective tissue and cells with the appropriate structural and mechanical properties [1,2]. Engel-

berg and Kohn [1] categorised these polymers into (a) poly(ortho esters), (b) poly(glycolic acid) (PGA) and poly(lactic acid) (PLA), (c) poly( $\beta$ -hydroxybutyrate) and copolymers with hydroxyvaleric acid, (d) poly( $\epsilon$ -caprolactone) (PCL), (e) polyanhydrides, (f) poly(trimethylene carbonate), and (g) polyiminocarbonates.

In order to promote tissue formation, the above materials must be processed into a form that can provide the appropriate spatial and temporal cues and signals that would enable the engineered tissue to develop into a fully functional tissue [2]; such 'scaffolds' must be mechanically sound, and their strength and stiffness should ideally approach that of the tissue it is to replace. The scaffold can take the form of films, beads, or foams, which may be used as cavity fillers to replace diseased tissue that has been removed during tumour

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resection, for example. Numerous scaffold fabrication techniques have been reported in the literature, many of which yield structures with varying degrees of microporosity. These include, for example, solution, gel and solvent casting, high-pressure gas foaming, lyophilisation, phase separation and emulsion templating. When combined with particle leaching methods, the porosity of these structures can be increased still further.

Before a scaffold can be considered for use as a substrate for cell culture, its surface properties must first be properly characterised and optimised. Thin films are often used for this purpose in biomaterials research, and to determine the effect of surface topography and chemical modifications on cell growth. Miller et al. [3], for example, produced a solvent cast poly(D,L-lactic acid) (PDLA) films with a micro-patterned surface topology that was transferred from a silicon wafer. Pattern widths or spacings of 10–20  $\mu\text{m}$  were found to be optimal for the alignment of Schwann cells (size 5–10  $\mu\text{m}$ ). Stavridi et al. [4] studied the effect of micro-patterned PCL films on platelet adsorption and found that unmodified PCL showed good blood compatibility whereas the patterned surfaces attracted more platelets and induced faster coagulation.

Lu et al. [5] cast poly(DL-lactic-co-glycolic acid) (PLGA) onto glass coverslips and reported that such films were promising substrates for the transplantation of retinal pigment epithelium regardless of the copolymer ratio. Ishaug-Riley et al. [6] spin cast a series of degradable polymer films for use in studies of human articular chondrocyte adhesion and proliferation. The chondrocytes attached and proliferated on all the biodegradable FDA-approved materials, including poly( $\epsilon$ -caprolactone), although chondrocyte adhesion was reduced on PCL and L-PLA films compared to PGA films. Calvert et al. [7] cast PCL, PLGA, and blends of the two polymers, onto glass microscope slides and studied the transplantation of osteoblasts. Of the two homopolymers, PLGA was found to be osteoconductive whereas PCL was less capable of retaining the osteoblast phenotype. And while the polymer blends exhibited superior osteoconductive properties compared to homopolymers, blends of PCL and PLGA that incorporated 10% PCL were considered to be compatible whereas those with 40% PCL were not. Nevertheless, PCL has found application in a number of medical devices concerned with drug delivery, and is actively being considered for applications where slower rates of degradation are desirable, for example, as a coating for urethral stents [8] and in musculoskeletal tissue engineering [2].

Two methods of producing PCL films have been presented in recent publications: solvent casting of polymer onto a Petri dish and spin casting onto glass coverslips, each of which can introduce large differences in terms of molecular conformation, surface chemistry,

surface energy, surface topology, porosity and pore size. Polar solvents are also widely used to remove surface contamination from polymeric materials that have undergone surface treatment, a process that may also give rise to significant changes in the surface energy and contact angle hysteresis of the surface [9]. Hitherto, researchers have not reported any differences between the two surfaces of the resulting cast films.

In this study, we set out to fabricate identical poly( $\epsilon$ -caprolactone) films cast from one of four different solvent systems: chloroform, tetrahydrofuran, acetone, and ethyl acetate. Each set of films was evaluated in terms of dynamic contact angle, surface microstructure and chemistry, and the ability of fibroblasts to adhere and proliferate on their surfaces.

## 2. Materials and methods

### 2.1. Poly(caprolactone) film fabrication

Poly(caprolactone) [Aldrich (Mw 80 K)] was used as received. The solvents used in this study were chloroform (Analytical reagent from VWR International), tetrahydrofuran (99.7% GC, HPLC grade from BDH Chemicals Ltd., Poole, England), acetone (99.8% GC, HPLC grade from BDH Chemicals Ltd., Poole, England), and ethyl acetate (99.5+%, urethane grade from Eastman Chemical).

The PCL film was manufactured using a modified solvent casting technique. Briefly, 2.00 g of PCL granules were dissolved in 40 ml of specific solvent. In case of acetone and ethyl acetate, a warm water bath (40°C) was used to aid the dissolution of the granules. The solution was cast into a glass Petri dish (diameter 90 mm), which was covered with a lid (diameter 100 mm) and placed in a fume hood at room temperature for a slow evaporation. The dried film was collected and vacuum dried for 48 h. The thickness of the resulting films varied according to the solvent used, and ranged from  $620 \pm 20 \mu\text{m}$  (chloroform) to  $1240 \pm 50 \mu\text{m}$  (ethyl acetate). Rectangular specimens, approximately  $6 \text{ mm} \times 7 \text{ mm}$ , were cut with a scalpel blade. The specimens were washed in deionised water and then dehydrated in a series of alcohol solutions (70, 80, 90, 95, 100, 100, 100 v/v%). The alcohol dehydrated PCL film specimens were vacuum dried for 48 h. All the samples were stored in desiccators until use.

The PCL films used in this study were handled and processed with due consideration for the differences between the polymer surfaces. The surface that had been in contact with glass during casting was denoted the SG surface, while the other, which had been exposed to the gas phase, a mixture of air and residual solvent vapour, was denoted the SA surface (Fig. 1). For the purpose of dynamic contact angle measurements, two vacuum-

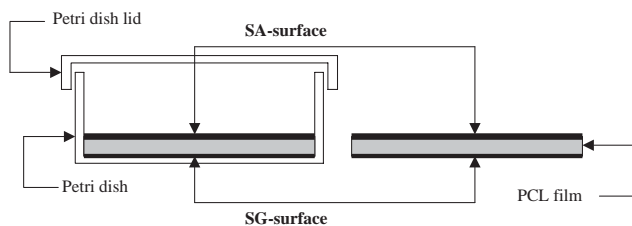


Fig. 1. Schematic diagram of the Petri dish arrangement used to cast the polymer films. SA denotes surface–air interface; SG denotes surface–glass interface.

dried films were glued together using a viscous PCL paste made from chloroform such that an identical surface, either SA or SG, was exposed on both sides.

## 2.2. Contact angle measurements

The advancing and receding contact angles for three replicate films of PCL were determined using a Dynamic Contact Angle Tensiometer (CDCA 100, Camtel Ltd, Royston, Herts, UK) at  $22 \pm 0.5^\circ\text{C}$  as reported by Jones et al. [8]. Briefly, each sample was attached to a microbalance and immersed into, and retracted from, the wetting medium (deionised water) at a rate of  $60 \mu\text{m s}^{-1}$ . The wetting force at the solid/liquid/vapour interface was automatically recorded via the electrobalance as functions of both time and immersion depth, and was converted into both the advancing and receding contact angles by the software supplied with the instrument. The static contact angle of the glass surface of the Petri dish was measured from digital photographs of a drop of deionised water in direct contact with the surface.

## 2.3. Scanning electron microscopy (SEM)

The surface microstructure was observed with the aid of a Field Emission Scanning Electron Microscope (FE-SEM) (LEO 1550, Cambridge, UK). Briefly, the sample film was coated with chromium (2 min and about 50 nm thick) under 125 mA. The coated sample was placed in the vacuum chamber of the FE-SEM and viewed at a voltage of 5 kV.

## 2.4. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra of the sample were generated by a Thermo Nicolet Nexus<sup>TM</sup> FTIR (Cambridge, UK) controlled by OMNIC software Version 6.1a. Briefly, the specimen was mounted onto a SMART OMNI-Sampler connected to the FTIR Nexus. The machine was operated under the experiment ATR-GE and the

spectra were obtained by accumulating 32 scans in the range  $600\text{--}4000 \text{ cm}^{-1}$  with a resolution of  $4 \text{ cm}^{-1}$ .

## 2.5. Cell adhesion and proliferation

L929 mouse fibroblasts were resuspended at a concentration of  $5 \times 10^4 \text{ cells ml}^{-1}$  in Gibco's 199 medium plus 5% Fetal Calf Serum (FCS) and antibiotics. 1 ml ( $5 \times 10^4$ ) of cell suspension was added to each material and a tissue culture polystyrene (TCPS) control in a 24 well plate.

The cell/material constructs were incubated for 2 and 6 days at  $37^\circ\text{C}$ , 5%  $\text{CO}_2$ . Viable cell adhesion was quantified using an LDH assay (Promega, UK), the value of LDH found in medium only, attributable to the presence of FCS, having been subtracted from all readings prior to quantification of viable cell adhesion to the PCL film. The PCL film was removed from the original culture wells prior to performing the LDH assay to ensure that only those cells that had adhered to the films were taken into account.

## 3. Results

Fig. 2 shows the contact angles of the solution cast PCL films. Chloroform cast PCL film had advancing contact angles of  $85.08 \pm 0.40^\circ$  and  $93.32 \pm 0.26^\circ$  on the SG and SA surfaces, respectively ( $n=3$ ). The SA PCL surface had a contact angle  $8^\circ$  higher than the SG PCL surface. THF cast PCL film recorded significantly higher values ( $p < 0.05$ ) for advancing contact angle ( $105.83 \pm 0.36^\circ$  and  $101.72 \pm 1.29^\circ$ , respectively). The difference of the advancing contact angles between the SG and SA surfaces was  $4^\circ$ . Likewise, the values for advancing contact angle for the acetone cast PCL film were significantly higher ( $p < 0.05$ ) than for film processed in chloroform ( $101.69 \pm 1.41^\circ$  and  $96.84 \pm 1.43^\circ$ , respectively). Here, the difference between SG and SA was about the same ( $4^\circ$ ). The values recorded for ethyl acetate cast PCL film were  $88.78 \pm 0.57^\circ$  and  $103.27 \pm 1.28^\circ$ , respectively. This solvent introduced the biggest difference between the SG and SA surfaces in terms of advancing contact angle ( $14^\circ$ ). The receding contact angles of the solvent cast PCL films were in the range  $25\text{--}35^\circ$ , with the exception of the SA surface of the chloroform cast PCL film, which had a receding contact angle of  $47^\circ$ , a difference of  $14^\circ$  between the two surfaces. The static contact angle of the glass surface of the Petri dish was  $26.48 \pm 0.71^\circ$  ( $n=4$ ), indicating that the surface was hydrophilic in nature.

Fig. 3 shows the hysteresis in contact angle exhibited by PCL films cast from different solvents, the degree of hysteresis being both surface and solvent dependent. The magnitude of the hysteresis exhibited by films cast from hydrophobic solvents ( $\text{CHCl}_3$ ; Et-Ac) was less

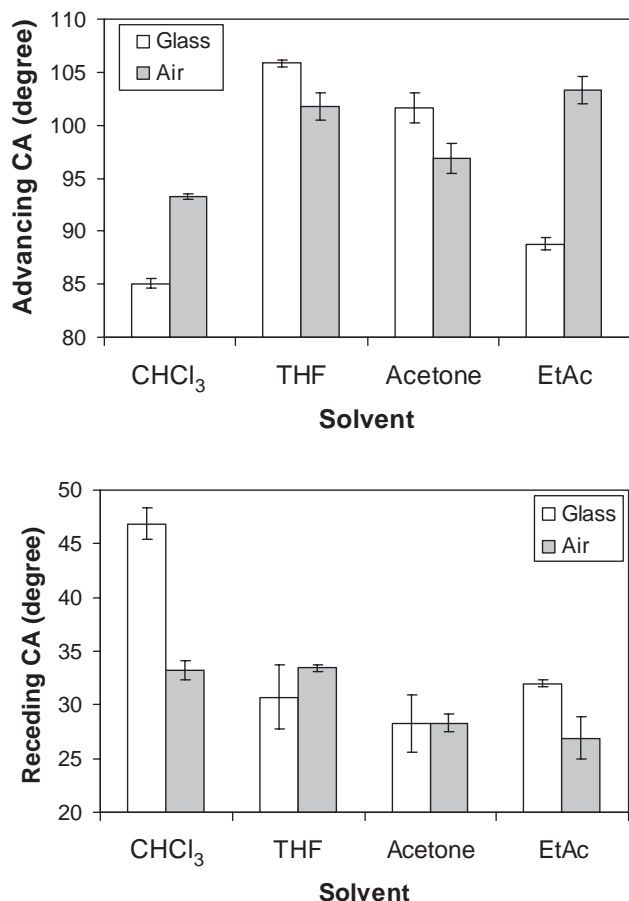


Fig. 2. Effect of solvents and interfaces on the contact angle of solution cast PCL films (CHCl<sub>3</sub>—chloroform; THF—tetrahydrofuran; EtAc—ethyl acetate). Again, SA and SG denote the surfaces in contact with air and glass, respectively, during casting.

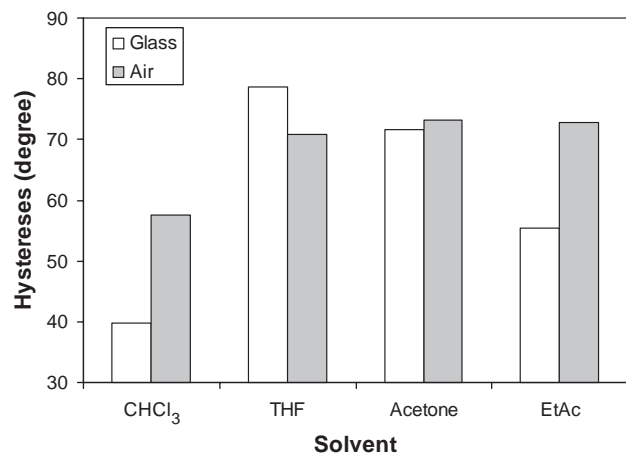


Fig. 3. Contact angle hysteresis on the respective surface as a function of solvent system used (CHCl<sub>3</sub>—chloroform; THF—tetrahydrofuran; EtAc—Ethyl acetate).

than that for the hydrophilic solvents, however the difference between the SA and SG surfaces was greater (~18° as opposed to 8° for THF). Films cast from

acetone, for example, a hydrophilic solvent with the lowest boiling point, exhibited only a 2° difference in contact angle hysteresis between the SA and SG surfaces. Interestingly, differences in surface morphology of the films did not appear to have much influence on contact angle hysteresis.

Fig. 4 shows the surface morphology of the solution cast PCL films. The SA surface of the chloroform cast film was smooth but uneven, with visible cracks in places. In contrast, the SG surface was rough with visible pores, and was characterised by discrete, spherule-like aggregations (diameters from 80 to 140 μm) which occupied the entire thickness of the film. The spherules were partially fused together, resulting in irregular voids on the surface.

PCL films cast from tetrahydrofuran (THF) exhibited rough surface morphologies on both sides. The SA surface appeared less porous than the SG surface. As with chloroform, the surface of the polymer appeared to be made up of fused aggregates (diameter from 20 to 70 μm). In contrast to PCL films cast from chloroform, however, a substantial number of the aggregates were not round but were elongated and partially fused together, leaving traces of narrow voids on the surface.

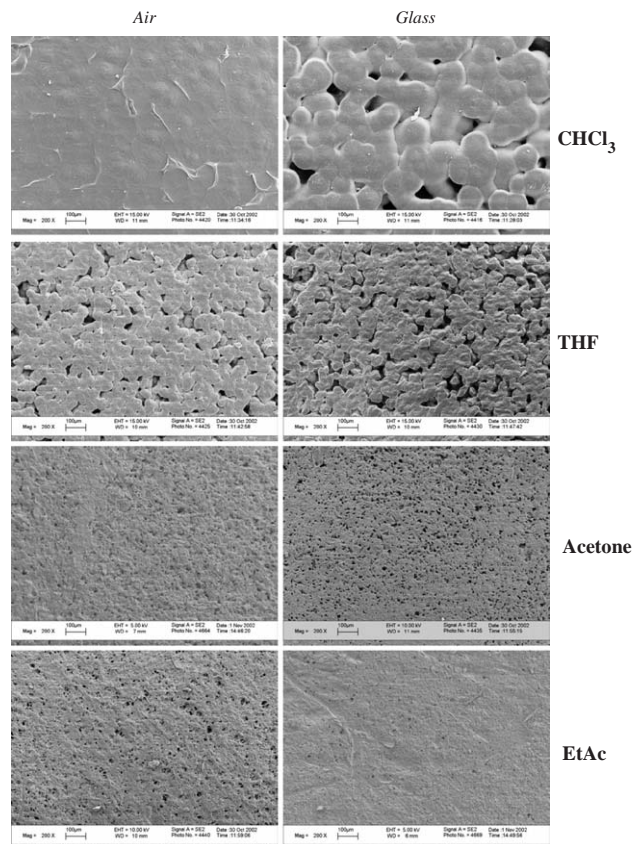


Fig. 4. Effect of solvents and interface on the surface morphology of solution cast PCL films (CHCl<sub>3</sub>—chloroform; THF—tetrahydrofuran; EtAc—ethyl acetate). The scale bar shown in each micrograph is 100 μm in length (×200 magnification).

The surfaces of the acetone cast PCL films also exhibited rough surface morphologies on both SG and SA aspects, however the SA surface was somewhat less porous than the SG surface (Fig. 4). Here, the PCL film comprised numerous filamentous PCL aggregations, which were interconnected by PCL fibres. The porous SA surface of ethyl acetate cast PCL films also comprised interconnected filamentous PCL aggregations. Interestingly, the SG surface appeared less porous and smoother than the SA surface.

Fig. 5 shows the ATR-FTIR spectra of the solvent cast PCL films. The PCL bulk material itself had characteristic absorption bands at  $1170\text{ cm}^{-1}$  (strong) and  $1180\text{ cm}^{-1}$  (shoulder). Solution casting shifted those peaks by  $10\text{--}1180\text{ cm}^{-1}$  and  $1190\text{ cm}^{-1}$ , respectively. The SA surfaces of the chloroform and THF cast PCL films showed similar patterns of the ATR-FTIR absorptions. Both recorded a strong absorption at  $1190\text{ cm}^{-1}$  and a broad shoulder at  $1180\text{ cm}^{-1}$ . The SA surfaces of the acetone and ethyl acetate cast PCL films possessed similar patterns of the ATR-FTIR absorptions, both

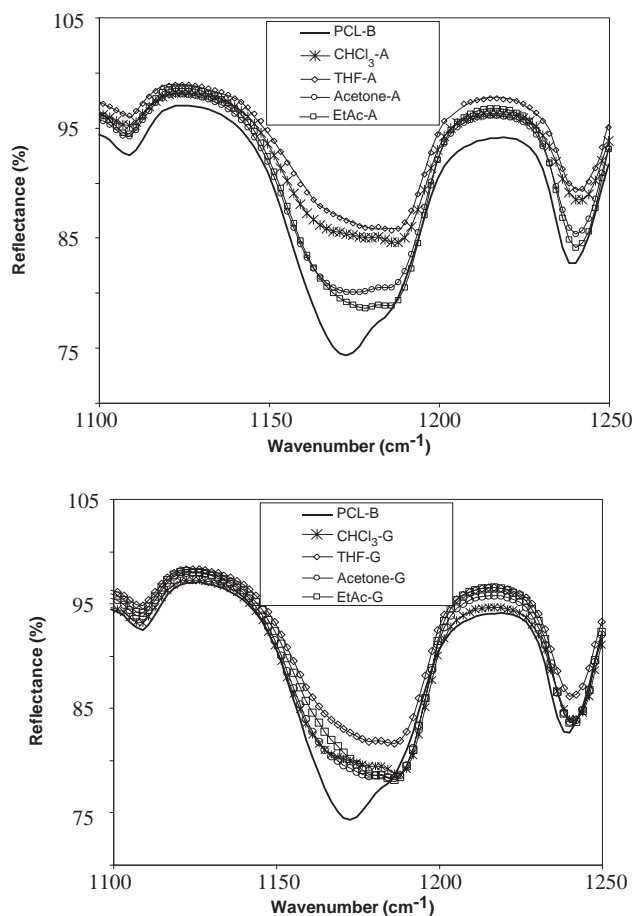


Fig. 5. ATR-FTIR spectra of the solvent cast PCL films ( $\text{CHCl}_3$ —chloroform; THF—tetrahydrofuran; EtAc—ethyl acetate). The surfaces A and G denote the surfaces in contact with air and glass, respectively, during casting. PCL-B denotes the result for the bulk PCL material in the form of granules as supplied by Aldrich.

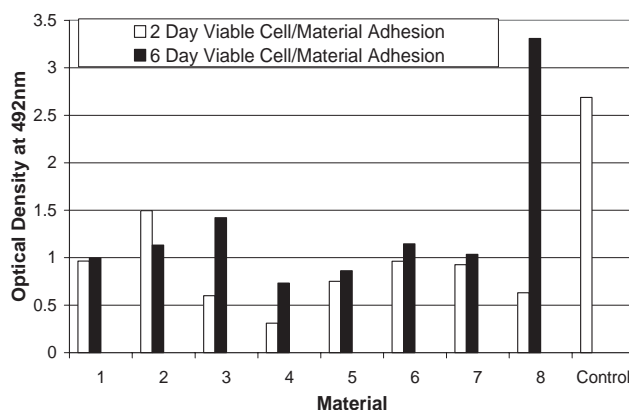


Fig. 6. Cell adhesion onto the solution cast PCL films. (1) PCL- $\text{CHCl}_3$ -Air (SA); (2) PCL- $\text{CHCl}_3$ -Glass (SG); (3) PCL-THF-Air (SA); (4) PCL-THF-Glass (SG); (5) PCL-Acetone-Air (SA); (6) PCL-Acetone-Glass (SG); (7) PCL-Ethyl acetate-Air (SA); and (8) PCL-Ethyl acetate-Glass (SG).

registering a strong absorption at  $1180\text{ cm}^{-1}$  and a fairly strong shoulder at  $1190\text{ cm}^{-1}$ . Compared to the SA surface, the SG surfaces were quite similar in ATR-FTIR. All showed a strong absorption at  $1190\text{ cm}^{-1}$  and a broad shoulder at  $1180\text{ cm}^{-1}$ .

Fig. 6 shows the cell adhesion results at 2 and 6 days. The 2-day results indicated that sample No. 2 was the most conducive to fibroblast adhesion whereas sample No. 4 was the worst. The numbers of cells adhering to the each surface after 2 days in an ascending order was as follows:

No.4 < No.3 and No.8 < No.5 < No.1, No.6,  
and No.7 < No.2.

After 6 days in culture, the surface most conducive to fibroblast adhesion and growth was sample No. 8 while sample No. 4 was the worst. The numbers of cells adhering to the surface at 6 days an ascending order was as follows:

No. 4 < No. 5 < No.1 < No.7 < No.2,  
and No.6 < No.3 < No.8.

With the exception of sample No. 8, the numbers of cells adhering to the above samples did not exceed the controls.

#### 4. Discussion

The major findings in this study are the variations in contact angle and surface morphology, differences which we attribute to the solvent systems used in each case. Jones et al. [8] presented the results of a study of PCL films cast on glass Petri dishes. The solvent they used was dichloromethane. The advancing contact angle of the resulting PCL films was recorded as  $89.5 \pm 1.2^\circ$ ,

which is comparable to the average of the advancing contact angles (SA and SG surfaces) reported here ( $89.2^\circ$ ). Interestingly, Jones et al. [8] did not distinguish between the two surfaces. The SEM morphology described in their report was also broadly similar to that described here. Again, these authors did not note any differences between the two surfaces.

PCL films have also been reportedly spin cast onto glass coverslips [6,7,10]. Spin casting has the advantage of casting smaller amounts of degradable polymer. The static contact angle of PCL films produced by this method has been reported as  $78 \pm 2^\circ$  [6],  $95 \pm 1^\circ$  [10], and  $73 \pm 2.5^\circ$  [7], however no explanation was given for these differences and any related morphological changes.

The results of the contact angle analysis for SA and SG surfaces show a clear relationship with solvents used, with hydrophobic solvents such as chloroform and ethyl acetate showing greater differences between SA and SG surfaces in terms of advancing contact angle (Fig. 2). Films cast from hydrophobic solvents also had higher advancing contact angles for the SA surface than for the SG surface. In contrast, films cast from hydrophilic solvents like acetone and tetrahydrofuran had higher advancing contact angles for the SG surface than for the SA surface, although these differences were somewhat less.

The choice of solvent also influenced the degree of contact angle hysteresis. Factors responsible for contact angle hysteresis of a polymer surface include surface contamination, heterogeneity of the surface structure, reorientation or mobility of the surface segment, swelling, and deformation [9,11]. Surface roughness is also thought to be a factor, however in our case the smooth SA surface of chloroform cast PCL film showed greater contact angle hysteresis than the rough SG surface (Fig. 3). Here, the solubility of the solvent appeared to be the dominant factor, as this correlated with the solvent induced filamentous and granular surface structure. Solvent contamination was not considered to be the main factor responsible for the change in contact angle hysteresis, since all the samples were dehydrated in series ethanol and vacuum dried for 48 h beforehand. Had there been any such contamination, the differences in contact angle hysteresis between the SA and SG surfaces would have been negligible. In fact, the films cast from hydrophobic solvents were more hydrophilic. Nor was the difference in the boiling point of each solvent thought to be a factor, since films cast from acetone, a hydrophilic solvent with the lowest boiling point, exhibited almost no difference in contact angle hysteresis between the SA and SG surfaces. Films cast from hydrophobic solvents, on the other hand, exhibited large differences in contact angle hysteresis between the two surfaces (Fig. 7), despite their significantly different boiling points ( $61^\circ\text{C}$  and  $77^\circ\text{C}$  for chloroform and ethyl acetate, respectively).

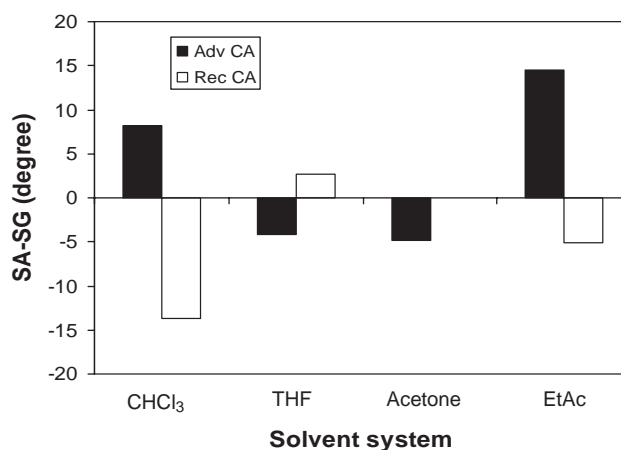


Fig. 7. Solvent-induced surface difference between the SA SG surfaces expressed in terms of the difference in contact angle (CHCl<sub>3</sub>—chloroform; THF—tetrahydrofuran; EtAc—ethyl acetate). SA and SG denote the surfaces in contact with air and glass, respectively, during casting.

We believe that the dissolution and subsequent phase separation of PCL molecules after casting was the most significant factor in determining the surface properties of the cast films. If we assume that the PCL molecules [1] are completely dissolved in both hydrophobic and hydrophilic solvents at the outset, it follows that they must aggregate in different ways. When the solution is cast into a Petri dish, the PCL molecules assemble according to the (hydrophilic) properties of the glass surface (contact angle  $26.48 \pm 0.71^\circ$ ), forming a laminated surface structure, which could explain the differences between the SA and SG surfaces (see Fig. 8). With hydrophobic solvents, the SA surface tended to be relatively hydrophobic, and so the advancing contact angle for the SA surface was always higher than that for the SG surface. With hydrophilic solvent cast PCL film, the SA surface tended to be more hydrophilic. The reversal in sign of the advancing contact angle (SA-SG) shown in Fig. 7 could have been due to the SA surface being more hydrophilic, which, in turn, depends on the hydrophilicity of the glass and solvent used in each case.

The absolute value of the advancing angle for each PCL film depended on the nature of the solvent and interface used for film formation, in particular, the surface area, the density of the hydrophilic functional groups, and the degree of surface roughness. Differences in surface roughness might explain the large difference ( $\sim 8^\circ$ ) in advancing contact angle detected between the SA and SG surfaces for the chloroform cast PCL films. Although similar in appearance to the chloroform cast PCL film, the ethyl acetate cast PCL film had the least porous SG surface, resulting in the largest gap ( $\sim 14^\circ$ ) in advancing contact angle between the SA and SG surfaces (Fig. 7). It is not clear either the extent to which differences in the size of the aggregates present in

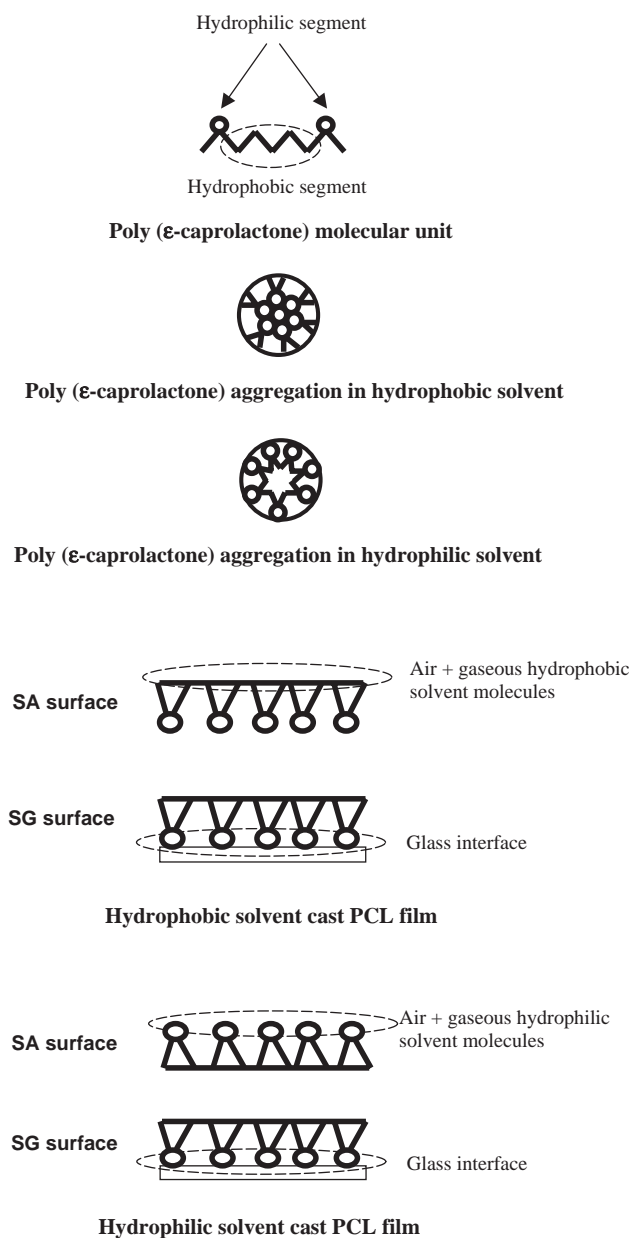


Fig. 8. Cartoons depicting the dissolution and aggregation of PCL molecules, and the formation of PCL films in different solvent systems.

chloroform and tetrahydrofuran cast PCL films contributed to the differences in contact angle. It would be of interest, therefore, to investigate further the relationship between surface porosity and dynamic contact angle.

The ATR-FTIR spectra for the SG surfaces were broadly similar, regardless of the solvent used, which suggests that the surfaces were the same chemically and supports our hypothesis that a hydrophilic glass surface initiates the aggregation of PCL molecules in a laminar fashion during phase separation. The reasons for the subtle variations in ATR-FTIR spectra for the SA surfaces, however, are complex and might involve many factors, for example, molecular interactions, particle size, porous structure, and perhaps surface roughness.

For the purpose of discussion, let us divide the spectra into two groups: (A) acetone and ethyl acetate and (B) tetrahydrofuran and chloroform. Group (A) solvents resulted in partial dissolution whereas group (B) solvents resulted in complete dissolution. The films in Group A exhibited a more filamentous morphology whereas those in Group B were comprised of particulates. The filamentous structure might be due to the poor solubility of PCL in certain solvents and the process of phase separation that takes place after casting. The faster nucleation that takes place with the poor solvent systems suggests that the PCL molecules might not have the same freedom to interact intramolecularly as in the case of the better solvents, forming a filamentous structure as a result. The better the solvent, the more likely it is that the PCL molecules will have more freedom to aggregate intramolecularly. When PCL was dissolved in chloroform, for example, the solution was clear and yielded translucent films. PCL films cast from tetrahydrofuran also had very few visible nucleation nodes.

The FTIR spectra suggest that these differences might be partially responsible for the observed changes in advancing contact angle. The characteristic absorption bands at 1170, 1180, and 1190  $\text{cm}^{-1}$  indicate that the ether bonds (C–O–C) of the PCL molecules might aggregate in different ways. The fact that none of the absorption bands correlated with the amorphous phase of PCL (unpublished data) suggests that these features may be attributed to differences in the structure of the crystalline phase of the samples rather than the degree of crystallinity.

The cell culture results indicated that the SG surface of the ethyl acetate cast PCL film (sample No. 8) is the most promising substrate for fibroblast adhesion and proliferation. Overall, fibroblasts adhered and grew better on the smooth surfaces with lower surface tensions. Fibrous samples with the finest pore structure were by far the most favourable for the attachment and proliferation of fibroblasts. Fibroblasts have previously been shown to prefer hydrophilic surfaces [11]. Lee et al. [12] observed that the cell adhesion and growth decreased gradually with increasing micropore size of the membrane surfaces. Among the three micropore sizes studied ( $\varnothing$  0.2, 1.0, and 5.0  $\mu\text{m}$ ), fibroblast preferred to adhere and grow on corona-treated polycarbonate membrane surfaces with a micropore size of  $\varnothing$  0.2  $\mu\text{m}$ . This result is consistent with the present study in that the pores of sample No. 8 were the finest (apart from the SA surface of the chloroform cast PCL film, which was non-porous). Fibroblasts adhered and proliferated more readily on the more hydrophilic SG surface as opposed to the SA surface of the ethyl acetate cast PCL film, in agreement with Lee et al. [12]. Likewise, Van Kooten et al. observed that fibroblasts proliferated better on grooves that were 2 and 5  $\mu\text{m}$  in

width than on grooves 10  $\mu\text{m}$  wide [13]. The fibroblasts used in this study also appear to be sensitive to the size of the pores present in the PCL film surfaces, as is evident from the outstanding performance of the SG surface of the ethyl acetate cast PCL film. Changes in surface chemistry, pore size and contact angle that take place over the 6-day in vitro culture period might also explain the increase in cell density seen with this sample.

## 5. Conclusions

In this paper, we present data which show the extent to which opposite surfaces of the same polymer film differ when cast onto a glass substrate from different solvents. By comparing the contact angles, surface morphology, surface chemistry and biocompatibility of PCL films cast from four different solvents, we have shown that the process of casting onto a glass substrate yields thin membranes, the properties of which depend on the nature of the solvent used and the surface properties of the substrate on which the films are cast.

With hydrophobic solvent systems, the advancing contact angle for the surface exposed to atmosphere during casting was higher than that for the surface in contact with the glass Petri dish; in the case of hydrophilic solvent systems, the opposite was the case. And while the ATR-FTIR spectra for all surfaces in contact with glass were similar, regardless of the solvent used, the spectra for the exposed surfaces were all different. The two surfaces also showed morphological differences in terms of the aggregates that formed during phase separation and the microporosity of the surface. While poor solvent systems (partial dissolution at room temperature) yielded filamentous PCL structures, good solvent systems (complete dissolution at room temperature) produced particulate structures.

Preliminary cell culture experiments carried out with the PCL films showed that fibroblasts grew well on all surfaces regardless of the solvent used, although the rates of adhesion and proliferation were not as great as on tissue culture plastic controls. Of all the surfaces examined in this study, the cells favoured the SG aspect of ethyl acetate cast PCL films, the surface of which had the finest pore size and relatively low contact angle.

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