Titanium Dioxide-Polycaprolactone Composite Films...

Simple One step Method to Produce Titanium Dioxide-Polycaprolactone Composite Films
with Increased Hydrophilicity, Enhanced Cellular Interaction and Improved Degradation for
Skin Tissue Engineering¹

Phong A. Tran ^{a,*}, Dhee P. Biswas ^a and Andrea J. O'Connor ^a

^a Department of Chemical and Biomolecular Engineering, The Particulate Fluid Processing
Centre, The University of Melbourne, Victoria 3010, Australia

* corresponding author, email: tran.p@unimelb.edu.au, (61)90355153 (ph), (61) 383444153
(fax); Department of Chemical and Biomolecular Engineering, The University of Melbourne,
Victoria 3010, Australia

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Abstract

There is a significant interest in using synthetic polymers, such as polycaprolactone (PCL), in engineering skin to avoid the need for donor sites with autografts, immunological rejection issues with allograft and reproducibility issues with using natural polymers. PCL is promising as it is a U.S. Food and Drug Administration - approved biodegradable polymer with good mechanical properties. However, its hydrophobic nature is not optimal for cellular interaction and biodegradation in skin tissue engineering. In this study, titanium oxide-PCL composite films were prepared using an *in situ*, one step synthesis method. Titanium dioxide (TiO₂) was introduced to improve the wetting properties of the hydrophobic polymer and so enhance the cell-material interactions and material biodegradation to be more suitable for skin regeneration. Results showed that the simple synthesis method produced nano- and sub-micron TiO₂ particles well dispersed within the PCL matrix. Spin-coated composite films showed increasing hydrophilicity with increasing concentration of TiO₂. Degradation of the composite films and pure PCL films were compared using gel permeation chromatography of the films after 14-day-immersion experiments. Molecular weights of PCL after immersion were found to steadily decrease by up to ~65% with increasing concentration of TiO₂. Rates of water penetration into the composite films were found to increase with the concentration of TiO₂ and correlate to the molecular weight decreases observed. *In vitro* experiments with fibroblasts demonstrated enhanced cell adhesion and proliferation on the composite films. This synthesis method therefore provides a simple means of tuning the wetting properties of hydrophobic polymers to enhance their cellular interactions, as well as tuning their biodegradation properties to suit applications such as skin tissue engineering.

Keywords: Composites, Sol-gel methods, Cell-material interactions, Hydrophobic polymer, Polymer degradation, Skin tissue engineering.

1. Introduction

Many synthetic polymers, such as poly(lactic-co-glycolic) acid and polycaprolactone (PCL), have been extensively studied as potential scaffold materials for tissue engineering. The use of a well characterised synthetic material has potential advantages for such applications including avoiding the need for donor tissue with autografts and immunological rejection with allografts, as well as supply and reproducibility challenges that can occur in using natural polymers. For skin tissue engineering, these polymers serve as the scaffold supporting cell functions and resisting contraction to allow the tissue to regenerate. Ideally, natural tissue is formed and fills the space and function of the polymer matrices that eventually degrade under the environment created by biological processes. Therefore, the matrices need to support functions (such as adhesion, proliferation and differentiation) of appropriate cells (such as fibroblasts) and have mechanical and degradation characteristics suitable for skin regeneration applications. However, the hydrophobic nature of many polymers like PCL makes them non-ideal for adsorption of important adhesive proteins (such as fibronectin, vitronectin) in their native conformation. The hydrophobic surfaces tend to cause protein denaturation and subsequent foreign body reactions to the implants *in vivo*. This in turn limits the functions of adherent cells including fibroblasts [1-4]. This hydrophobic nature of polymers like PCL also results in very slow degradation, often requiring one year or more for complete degradation in the case of PCL [5-7]. In skin tissue engineering applications where tissue formation takes place in the first several months following the surgery, a faster degradation rate is desirable.

Therefore, in this study we demonstrated that by simply altering the wetting properties of the model polymer PCL, we have (i) enhanced its interaction with fibroblasts and (ii) accelerated its degradation. PCL is a biodegradable polyester polymer synthesized by ring-opening polymerization reaction of ϵ -caprolactone [8]. Its biodegradability is attributed to non-

enzymatic hydrolysis of the ester bonds in the polymer chains in the physiological environment [9,10,5]. Reports in the literature have demonstrated methods such as hydrolysis using concentrated NaOH to make polymers such as PCL more hydrophilic for cell interactions [11-13]. However, these methods tend to only alter the wetting properties of the surface of the materials and can lead to etching; therefore they may not be ideal.

Here we use a sol-gel process to incorporate titanium oxide (TiO_2) into the PCL network to achieve changes to both the material surface and interior. TiO_2 is a passive, inert oxide that renders titanium and titanium alloys nontoxic toward several cell lines including osteoblasts [14,15] and fibroblasts [16,17]. TiO_2 has an interesting wetting property which can be tuned from super-hydrophobic to super-hydrophilic [18-20] by radiation (such as UV light) [21-23] and this process can be reversed by storage in the dark. Without radiation treatment, TiO_2 films often have water contact angles of from 50 to 70 degrees.

Sol-gel techniques are commonly used to synthesize TiO_2 via hydrolysis and condensation of precursors such as titanium alkoxides. The process consists of hydrolysis of the bonding between titanium and other groups (such as $\text{C}_4\text{H}_9\text{O}$ - groups in titanium butoxide) followed by condensation to form Ti-O bonds. With appropriate choice of solvents and conditions, this sol-gel process can be designed to proceed in the presence of a hydrophobic polymer matrix (such as PCL) to create a composite consisting of TiO_2 particles well-dispersed in a hydrophobic matrix using a one-pot synthesis.

In this paper, we present TiO_2 -PCL composite films developed using this simple, one-pot *in situ* synthesis approach. The new composite films are assessed in terms of their physicochemical properties, *in vitro* cell interactions and degradation.

2. Materials and methods

2.1. Preparation of composite films

Polycaprolactone (PCL, molecular weight ~90,000 kDa as given by supplier), was purchased from Sigma Aldrich (Castle Hill, NSW, Australia). N-butanol and chloroform (analytical grade) were obtained from Chem Supply (Gillman, SA, Australia). PCL was dissolved in chloroform to make a solution of 0.1g/mL by stirring for a few hours for complete dissolution. Titanium butoxide (Sigma Aldrich) was diluted to 0.1g/mL in butanol. To make composites, appropriate volumes of PCL solution and titanium butoxide solution were mixed uniformly using a magnetic stirring bar. Five different compositions were prepared to achieve final weight percentages of TiO₂ of 2.5%, 5.5%, 9.1%, and 13.5% in the composites (assuming 100% conversion of titanium butoxide to TiO₂).

Spin-coating was used to produce composite films convenient for testing. For this, glass coverslips (15mm in diameter, Thermo Scientific, NSW, Australia) and microscope glass slides (Thermo Scientific, NSW, Australia) were cleaned by immersing in 1% HCl at 50°C for 4 hours followed by rinsing in Milli-Q water (resistivity 18.2 MΩ·cm at 25 °C, Millipore, MA, USA), sonicating in a series of diluted ethanol (analytical grade, Chem Supply) solutions (30%, 50%, 70% and 95%) with a Milli-Q water rinsing step between each ethanol solution. This cleaning procedure is useful in removing contaminants on glass surfaces and increasing adhesion of composite films in the next step.

The air-dried coverslips and glass slides were used to spin-coat (at a speed of 100 RPM, Spincoat system, NSW, Australia) a layer (~ 0.1 to 0.3mm thickness) of composite materials from the PCL-Ti butoxide composite solutions. The spin coated films were air-dried for 2 days in a fume hood and UV-irradiated for 30 minutes for sterilization then stored in a sterile, closed container in the dark which has been shown previously to reverse any changes in wetting properties of TiO₂ caused by UV- treatment [21-23].

2.2. Material characterization.

Surface morphology of the composite films was studied using scanning electron microscopy in low-vacuum mode (Quanta ESEM) with an acceleration voltage of 10kV or 15kV without a conductive coating layer.

Surface topography at the nanometer scale of the composites was investigated using Atomic Force Microscopy (AFM, Asylum Research, Santa Barbara, CA, USA) with tips (Budget Sensors, Sofia, Bulgaria, less than 10nm in radius) in tapping mode.

Surface hydrophilicity and hydrophobicity of the composite films were evaluated by measuring static contact angles of water on the films (Rame-hart Std 100, NJ, USA). For this, 2 μ l drops of distilled water were placed on the films and contact angles were determined using an attached CCD camera after reaching stable values (no more than 2% variation in the values).

The chemistry of the composite films was studied using X-ray photoelectron spectroscopy (XPS, VG ESCALAB 220i-XL, VG Scientific) equipped with a monochromatic Al K α X-Ray source, which emitted photon energy of 1486.6 eV at 10 kV and 12 mA. Spectra were obtained at a step size of 1 eV (survey scans) or 0.1 eV (region/high resolution scans).

Tensile tests were conducted according to the ASTM D638 standard for tensile properties of unreinforced and reinforced plastics with thickness less than 14mm. The films were cut into dogbone shapes with the dimensions of 15mm x 2mm x t (t is the thickness of film, ranging from 0.1mm to 0.3mm) in the narrow section and were stretched at a speed of 2.5mm/minute using an Instron Micro Tester (Instron 5848 Materials Testing System, MA, USA) with a 2kN load cell.

Rates of water absorption were determined by monitoring weights of films immersed in Milli-Q water over a period of 120 hours.

Degradation of films was investigated by immersing the films in Dulbecco's Modified Eagle Medium (DMEM, supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin Streptomycin (P/S) all obtained from Sigma Aldrich) for 2 weeks at 37°C followed by analysis of molecular weight using gel permeation chromatography (GPC) in a Shimadzu system with three Phenomenex phenogel columns (500, 10^4 , and 10^6 Å porosity; 5µm bead size; Phenomenex). The commonly used cell culture medium DMEM was chosen because it contains important proteins and inorganic salts that are present in physiological environments with which implanted materials often interact. This medium was also used in subsequent biological testing in this study. The films were rinsed with Milli-Q water after the immersion experiment and dissolved in THF (HPLC grade, Chem Supply) and analyzed by GPC with a Wyatt Dawn F laser photometer operating at 90° coupled with a Waters 410 differential refractometer (for measurement of refractive index). The mobile phase was THF, flowrate was 1.0 mL/min, injection volume was 20 µL and column temperature was 30 °C. Astra software (Wyatt Technology Corp.) was used to process the data using the known dn/dc (rate of change of refractive index with concentration) value to determine the molecular weight.

2.3. Titanium release from composite films.

To study the possible dissolution of titanium from the composites and release into the surrounding environments in biomedical applications, an immersion experiment was conducted. For this, coated coverslips were placed in the same cell culture medium used above in the wells of a 24 well tissue culture-treated plate (BD Biosciences, CA, USA). Glass coverslips coated with PCL-only films were used as controls.

The plate was placed in an incubator (37°C, 5% CO₂) for a period of 72 hours, which is the longest duration of the subsequent biological tests. DMEM extracts were then collected and used to quantify amount of titanium released using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Agilent Technologies, Santa Clara, USA).

2.4. Cell adhesion on composite films.

To study the potential of using the composites for biomedical applications, mouse 3T3 fibroblasts (CCL-92, ATCC, VA, USA) were used for biological testing. This cell line has been widely used to test cytotoxicity and biocompatibility of materials for biomedical applications. Fibroblasts (population numbers between 5 and 8) were seeded in complete DMEM onto spin-coated coverslips in the wells of a 24 well tissue culture-treated plate (BD Biosciences) at a density of 50,000 cells per well. The plate was then placed in an incubator maintained at 37°C and 5% CO₂. Glass coverslips coated with PCL-only films were used as controls. After 4 hours, the cells were rinsed with phosphate buffer saline (PBS, Sigma Aldrich) twice, fixed in 4% para-formaldehyde (Sigma Aldrich), incubated with the nucleic acid fluorescent stain 4',6-diamidino-2-phenylindole (DAPI, Sigma Aldrich), rinsed again in PBS twice then imaged using an inverted fluorescence microscope (Olympus IX71, Olympus Australia, Victoria, Australia). Five random fields on each sample were imaged and the numbers of cells were counted (using Cell Counter in Image J, NIH, USA) and averaged.

2.5. Cell proliferation experiment.

Fibroblasts were seeded in complete DMEM onto the spin coated cover slips in the wells of a 24 well tissue culture-treated plate at a density of 2,500 cells per well. Glass coverslips coated with PCL-only films were used as controls. The plate was placed in an incubator (37°C, 5% CO₂) for 72 hours and cell proliferation was assessed using CellTiter 96® AQueous One Solution Cell Proliferation (Promega, NSW, Australia) reagent following the supplier's instruction. This MTS-based assay employs the tetrazolium compound which is bio-reduced by living cells [24] to a soluble, colored formazan product the intensity of which can be measured using a spectrometer (Varian Cary 50MPR, Agilent Technologies, Santa Clara, USA) at a wavelength of 490nm. Cell numbers were then calculated from the optical densities (ODs) using a pre-established standard curve. Cells were also fluorescently labeled

and imaged as described above. Lower cell seeding density compared to that used for the adhesion experiment was used to avoid the reduced growth rates expected as cell numbers approach confluence.

All experiments were implemented in triplicate unless otherwise indicated. Data are reported as mean $\pm$ standard deviation. Significant differences are reported for comparisons between samples that yielded $p < 0.05$ using the *Student* one-tailed *t*-test.

3. Results and discussion.

3.1. *In situ* formation and distribution of TiO₂ in the composites.

A uniform distribution of hydrophilic TiO₂ in the composite is desirable to improve its interaction with cells and its hydrolytic degradation. The composite films were examined under SEM to study the distribution of the TiO₂ phase. The images show that the composite films appear to be more rough and contain two distinctive phases (Figures 1(b), (c) and (d)) compared to plain PCL film (Figure 1(a)).

Fig.1 SEM images of the surface of PCL films incorporating increasing amounts of TiO₂: (a) 0 w/w %, (b) 2.5 w/w %, (c) 5.5 w/w %, and (d) 9.1 w/w %. Scale bars are 5 μ m

The bright clusters were identified as Ti-containing by using back scattered electron microscopy (BSE) images, which show contrast based on the atomic weights of elements present in the material rather than the topography of the material's surface. These clusters were later identified (using XPS) as titanium dioxide (TiO₂).

Fig.2 (a) and (b): SEM and BSE images of surface of a representative PCL film incorporating 5.5% TiO_2 showing co-location of bright spots indicating Ti-containing clusters. (C): SEM image of cross section of the composite film showing TiO_2 particle distribution. Scale bars are $5\mu\text{m}$

Figures 2(a) and 2(b) are SEM and BSE images of the same material area on a representative 5.5% TiO_2 -PCL film. The co-location of bright clusters on the SEM image and the BSE image indicate that these were likely particles of TiO_2 on and/or shallowly buried beneath the PCL surface. Together with the SEM image of the cross section (Figure 2C), it is clear that the inorganic phase (TiO_2) was relatively well dispersed in the organic phase (PCL). This dispersion characteristic is important for the potential application of these materials as the uniformity of the hydrophilic phase is expected to be beneficial for the material's interaction with mammalian cells, as well as the rate of water-sorption and hence hydrolytic degradation. The common synthesis method of mixing pre-formed inorganic particles in a polymer solution before casting is straightforward but often results in agglomeration of the particles in spite of extensive energy required for dispersing the particles. The synthesis method developed here using *in situ* formation of TiO_2 in the PCL solution overcomes the aggregation issue, resulting in composites having inorganic particles highly dispersed within the organic phase.

3.2. Surface topography of composite films.

To confirm the observation of rougher surfaces on composite films seen in the SEM images, AFM was used to investigate the topography of the representative 5.5% TiO_2 -PCL composite film and compare it to a PCL-only film. AFM results indicated that the composite film

appears to be rougher at the nano-and submicro-meter scales due to the presence of TiO_2 compared to the PCL-only film (Figure 3).

Fig.3 Topography and representative line profiles obtained from AFM data of PCL-only ((a) and (b)) and a 5.5% TiO_2 -PCL composite film ((c) and (d)). X and Y are axes in the plane of the samples' surfaces. Z is the axis perpendicular to this plane

3.3. Chemical state of titanium in the composites.

To determine the chemical state of the titanium in the composites, X-ray photoelectron spectroscopy was employed. A survey scan of the representative 5.5% TiO_2 -PCL composite showed peaks due to C, O and Ti, as expected (Figure 4).

Fig.4 The XPS spectrum of a representative 5.5% TiO₂-PCL composite showing the important peaks of C, O and Ti and high resolution scan (inset, with background subtracted) showing the positions of the characteristic peaks of Ti

Peaks of C at 285nm and O at 532nm are expected from the PCL polymer in the composites. A high resolution scan was used to identify positions of Ti 2p peaks and provided information about oxidation state of Ti (Figure 2 inset). The positions of Ti 2p^{1/2} and 2p^{3/2} peaks at 464 eV and 459 eV, respectively, suggest that Ti is in the form of TiO₂ [25]. The formation of TiO₂ was expected as a result of the hydrolysis and condensation of titanium butoxide in the presence of moisture in the air during spin-coating and air-drying. The same oxidation state of Ti was also determined from the composite after etching (~30nm deep) using an argon beam for 120 seconds on either side of the films and the centre of the cross-section of the films, implying that moisture was able to penetrate into the bulk of the films to react with titanium butoxide and/or its hydrolysis and condensation products.

3.4. Mechanical properties

The mechanical properties of the films were investigated using tensile tests. The Young's modulus of all composite films was found to be higher than that of PCL-only film (Figure 5(a)). Specifically, Young's modulus of the films was found to increase from 116±20 MPa for PCL-only films to 211±67 MPa for 2.5% TiO₂-PCL films. The value peaked at 403±43

MPa for 5.5% TiO₂-PCL films and decreased at higher TiO₂ concentration to 258±34 MPa (Figure 5(a)).

Fig.5 Young's modulus (a), elongation-at-break (b) and tensile strength (c) of the PCL-only and TiO₂-PCL composite films. * p< 0.05, n=3

The elongation-at-break of the films was also found to increase to 260±16% for the 2.5% w/w TiO₂ composite compared to 184±9% for PCL-only but decrease back to 160±17% at higher concentrations of TiO₂ (Figure 5 (b)). A similar trend to the Young's modulus was observed for the tensile strength of the films which increased from 1±0.07 MPa for plain PCL films to 1.9±0.2 MPa for 2.5% TiO₂-PCL films, 2.7±1.2 MPa for 5.5% TiO₂-PCL films and decreased to ~1.8 MPa for films with 9.1% and 13.5% TiO₂ (Figure 5 (c)) These tensile strengths are of the same order of magnitude as that of skin which has tensile strength of from 2.5 to 16 MPa [26].

Our observation of dependence of mechanical strength on the amount of TiO₂ in the composites is similar to some other studies which reported the effects of inorganic fillers on mechanical properties of polymers. The effect was observed as reinforcing at low concentrations of fillers due to the transfer of stress to the inorganic particles which have much higher stiffness compared to the polymer matrix [27]. But higher concentrations of fillers were found to be detrimental to the elasticity of the particulate-polymer composites in the case of particles not strongly bound to the polymer matrix [28-30]. This is likely the case

in our TiO₂-PCL composites in which the TiO₂ particles are not chemically bound to the PCL matrix.

3.5. Surface wetting properties of the composites.

Water contact angle measurements were conducted to confirm that the incorporation of TiO₂ particles into the hydrophobic PCL matrix increased its hydrophilicity. The results showed that the PCL-only film was relatively hydrophobic, as expected with a water contact angle of approximately 92° (Figure 6).

Fig.6 Static water contact angles on PCL-only and TiO₂-PCL composite films. * $p < 0.05$, $n=3$

Compositing with TiO₂ did make the film more hydrophilic as desired. As the amount of TiO₂ increased, the water contact angle of the films decreased to approximately 65° for the 13.5% TiO₂-PCL film. From this data, it can be concluded that TiO₂ particles were present not only within but also on the surface of all the composite films to directly contribute to the increase in hydrophilicity.

3.6. Water-sorption rates of the composites

Water absorption into the composite films was determined and compared with the PCL-only films. From the results, it can be seen that all the composite films, in particular the films with 5.5 w/w% TiO₂ or more were quickly saturated after immersing in water compared with films of PCL-only which had still not reached equilibrium after 120 hours (Figure 7).

Fig.7 Water-sorption of the PCL-only and PCL-TiO₂ composite films. The composite films had higher water-sorption rates leading to faster saturation while PCL-only film still had not reached saturation during the time period tested

The increase in water-sorption rates for the composites can be attributed to their increasing content of the hydrophilic TiO₂ particles. This enhancement in water penetration is expected to accelerate the hydrolytic degradation of the PCL matrix, as investigated in the following section.

3.7. Degradation of composite films

An important aim of the current study is to increase and regulate the degradation rate of the hydrophobic PCL material. After 2 weeks of immersion in DMEM, the degradation of PCL and PCL-TiO₂ composites was evaluated using GPC. A steady increase in the retention time of the polymer from the composites of with increasing TiO₂ content was observed. This indicates that the PCL molecular weight decreased faster upon immersion as the TiO₂ content was increased (Figure 8).

Fig.8 GPC traces of the films after 2 weeks' immersion in cell culture media

The PCL sample molecular weights (Mw) were estimated based on dn/dc (rate of change of refractive index with concentration) being 0.075 mL/g for PCL in THF [31]. The Mw of the PCL from the composites were lower than that of the immersed PCL-only film by 26%, 47%, 54% and 65 % for the composites with 2.5%, 5.5%, 9.1% and 13.5% TiO_2 , respectively. The 2 week - immersed PCL-only film had a similar retention time to that of the fresh PCL-only film, as expected from the slow degradation of PCL (~ 1 year or more to complete). The effect of the greatest decrease in Mw was also clearly visible for the composite with 13.5% TiO_2 , which disintegrated into small pieces at the end of the degradation experiment. It can be seen from these results that by incorporating TiO_2 into the PCL matrix, the slow degradation of this hydrophobic polymer can be readily changed to proceed at higher rates more suitable to skin engineering applications.

3.8. Titanium release from the composites.

The decrease in Mw of the polymer in the composites following 2 weeks' immersion is hypothesized to stem from water adsorption on or around the TiO_2 particles present close to the outer surfaces of the composites. This uptake is expected to facilitate the particles dislodging from the composites, creating pathways for further penetration of water and hence, hydrolysis of PCL chains. To test this hypothesis, the amounts of Ti present in the extraction media following immersion of the composites for 72 hours were measured. ICP-MS was

employed here because of its sensitivity for measuring metals in solution at small concentrations (in the parts-per-billion range for elements such as titanium). The results showed that all extracts from the composite films contained trace amounts of titanium and that the 9.1% and 13.5% TiO₂-PCL composites released significantly higher amounts of titanium than the 2.5% and 5.5% composites (Figure 9).

Fig.9 ICP-MS analysis of media extracts from composite films. * $p < 0.05$

The titanium in the composites was identified from XPS results to be in the oxidized form which is relatively chemically inert in the immersion medium. Therefore, the titanium detected in the media extracts was likely to still be TiO₂. The TiO₂ nano- and submicro-particles may be released from the surface of the PCL matrix, possibly supplemented by TiO₂ loosely bound to the PCL matrix that expanded under the pressure of penetrating water thus releasing the entrapped TiO₂. This is strongly supported by the SEM image of the 9.1 %TiO₂ composite film after the 2 week immersion test, showing pores which were likely to have been created by the release of TiO₂ particles (Figure 10).

Fig.10 SEM image of the 9.1%TiO₂-PCL composite surface after the 2 week immersion test.

It can also be noticed that the pore sizes in the films after immersion test are larger than the sizes of TiO₂ particles found before the test. This can be understood as the results of water penetration causing swelling of the polymer matrix

The release of TiO₂ from these composites into culture media is not expected to cause cytotoxicity, as it has been reported that micrometer and sub-micrometer TiO₂ particles were non-toxic to fibroblasts at concentrations up to 0.83 ppm [32]. The release was estimated to be less than 1% (w/w) of the total TiO₂ in the composites (based on the total weights of the composites) over the period of 72 hours and this is likely to continue beyond time period tested.

3.9. Cell adhesion to composite films.

Cell adhesion is an early, important process through which cells attach to the surfaces of implanted materials. This early interaction of biomolecules and cells with the material is strongly dependent on the material's surface properties, among which hydrophilicity is a key. All of the composite samples showed increased cell adhesion compared to the PCL-only sample (Figure 11).

Fig.11 Adhesion of fibroblast cells increases on composites with increasing TiO₂ content after 4 hours (a). Fluorescence images (taken with 10X objective, cell nuclei stained with DAPI) show cell densities on PCL films incorporating (b) 0 w/w % , (c) 2.5 w/w % , and (d)13.5 w/w % . TiO₂; * p<0.05, n=3

Cell adhesion on the 2.5%, 5.5% and 9.1% TiO₂-PCL samples did not differ significantly but was significantly lower than that on the 13.5% TiO₂-PCL sample. This increase in cell adhesion is correlated with the increase in hydrophilicity of the composite films.

3.10. Cell proliferation on composite films.

To study how the composite materials support fibroblast proliferation, mouse fibroblasts were cultured on the composite films, with PCL-only films as a control. The results showed that all the films support cell proliferation and that cell proliferation was increased somewhat on the higher TiO₂ content composite films relative to the PCL-only film (Figure 12).

Fig.12 Proliferation of cells after 72 hours' culture on PCL-only and TiO₂-PCL composite films (a). Fluorescence images (taken with 10X objective, cell nuclei stained with DAPI) show cell densities on PCL films incorporating (b) 0 w/w % , (c) 2.5 w/w % , and (d) 13.5 w/w % . TiO₂; * p<0.05, n=3

Cell numbers on the 5.5%, 9.1% and 13.5% TiO₂-PCL composite films were significantly higher than on PCL-only films. However, there was no significant difference in cell proliferation among the different composition TiO₂-PCL samples. Fluorescence microscopy images showed confluent cell layers on these composite films after 72 hours' culture. This observation is consistent with the known characteristic saturation density of approximately 50,000 cells/cm² for this cell line [33]. Our proliferation data (Figure 12) showed cell numbers of approximately 80,000 cells per composite film coated on 15mm glass coverslips or densities of 47,000 cells/cm². Therefore, it is likely that contact inhibition in the confluent cell layers on these proliferation-promoting composite samples resulted in the lack of significant difference in cell numbers as a function of TiO₂ content.

4. Conclusion

This study aimed to create composites of PCL with TiO₂ for potential use in skin engineering applications where faster degradation of the PCL matrix and better interaction with

mammalian cells are desired. We chose to change the wetting property by creating composites of PCL with hydrophilic TiO_2 . We presented a simple yet versatile one-pot synthesis method that induced hydrolysis and condensation of titanium butoxide inside a PCL matrix to achieve composites with a well dispersed inorganic phase in the organic phase. The TiO_2 -PCL composite films were found to have increased hydrophilicity, increased water-sorption rates and increased degradation. Importantly, adhesion and proliferation of mouse fibroblasts was enhanced on the composite films. Future work will include study of the tissue formation on the composite materials *in vivo* as well as their *in vivo* biodegradability.

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Author/s:

Tran, PA;Biswas, DP;O'Connor, AJ

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