Material Properties

Ibuprofen-loaded PCL meshes manufactured using rapid tooling for ocular orbital repair

Gean V. Salmoria a, b, *, Francesca Sibilia a, c, Izabelle M. Gindi b, Carlos R.M. Roesler b, Silvia Farè c, Maria C. Tanzic c

a CIMJECT Laboratory, Department of Mechanical Engineering, Federal University of Santa Catarina, 88040-900 Florianópolis, SC, Brazil
b Biomechanics Engineering Laboratory, University Hospital (HU), Federal University of Santa Catarina, 88040-900 Florianópolis, SC, Brazil
c Dipartimento di Chimica, Materiali ed Ingegneria Chimica “G. Natta”, Politecnico di Milano, Edificio 6, Piazza L. da Vinci 32, 20133 Milano, Italy

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A B S T R A C T

This study investigated the manufacture of resorbable polycaprolactone/ibuprofen (PCL/IBP) meshes by injection molding for application in ocular orbital repair. The pore dimension sizes used demonstrate that micro-porous meshes can be manufactured by injection molding using a prototype mold. The mechanical properties were observed to be dependent on the material composition and morphology. Lower stiffness, strength and elongation at failure were observed for the 8 mm pore sized samples. The PCL/Ibuprofen meshes initially showed a fast drug release but after 3 days the release was slow and controlled. The cytotoxicity test results of the PCL/Ibuprofen meshes indicated that the large initial quantity of Ibuprofen released was too high and resulted in cell toxicity. However, after this initial release, the PCL/Ibuprofen meshes showed a good interaction with the cells seeded on their surface. The presence of a low concentration of Ibuprofen does not negatively influence cell viability in culture.

1. Introduction

Orbital floor fractures are usually a consequence of mechanical trauma caused by traffic accidents, falls, and sports injuries [1,2]. Adequate medical treatment is critical to avoid complications due to these injuries, such as diplopia, enophthalmos, paresthesia, and aesthetical concerns [1,3,4]. Several types of surgical techniques and materials are used to reconstruct the orbital floor and restore the eye functionality by repairing the injuries from the trauma and relocating the globe into its correct position [5,6]. Polymeric implants, in particular, have received popularity due to their good processability, flexible geometric features, and the associated reduction in surgical morbidity [7–9].

Porous high-density polyethylene (HDPE) is an inert and non-resorbable polymer, which has been widely used for orbital floor reconstruction over the last 25 years. HDPE is available in plates of different sizes and thicknesses with pore sizes ranging from 100 to 200 μm [2,4,10]. The porous structure enables tissue ingrowth and fibrovascularization of the implant as well as reducing reactions to foreign bodies and capsule formation. However, problems associated with HDPE-based devices include adhesion of extra ocular muscle and orbital fibroadipose tissue to the implants. Furthermore, lower lid retraction and external scarring are common complications when non-resorbable polymers are used [11].

To overcome these problems, resorbable polymers have been proposed for the reconstruction of orbital floor fracture due to their controllable and predictable absorption kinetics [10,12,13]. Their main advantage is to provide support to the orbital structure under herniation forces during the initial healing phase [10,13]. Polycaprolactone (PCL) is a biodegradable polyester approved by the Food and Drug Administration (FDA) for use in the human body as implant material [14,15]. PCL-based scaffolds are good alternatives to repair cranial defects and bony contours in the craniofacial skeleton. Recent studies have shown that due to the mechanical properties similar to bone and to the slow degradation kinetics, this material triggers increased vascular ingrowth and osteoconductivity [16,17].

Besides the material properties, the success of reparative surgery also relies on the absence of severe inflammation and infection after the medical procedure. The use of implants loaded with drug delivery systems is a powerful strategy to minimize this problem.
Ibuprofen is a nonsteroidal anti-inflammatory drug. Its pharmacological effects are believed to be due to the inhibition of cyclooxygenase-2 (COX-2), which decreases the synthesis of prostanoids involved in mediating inflammation, pain, fever, and swelling [18,19]. In addition to its anti-inflammatory properties, ibuprofen has also shown good antimicrobial activity against clinically relevant strains. Furthermore, PCL loaded with ibuprofen has already been used for different applications with success in the biomedical field [20,21].

Injection molding enables the rapid development of polymeric implants and drug delivery devices [16] with complex geometries; also it offers elevated productivity using prototyping molds, known as rapid tooling [17–19] [22–24]. In this study, resorbable poly-caprolactone/ibuprofen (PCL/IBP) meshes were prepared by injection molding using rapid tooling. The structure as well as the physic-chemical, mechanical and biological properties of the meshes were characterized and evaluated for use in orbital floor healing.

2. Experimental

2.1. Materials

Poly(ε-caprolactone) pellets with 3 mm diameters (reference 440,744) were acquired from Sigma-Aldrich, USA. Weight average molecular weight (Mn) was found to range from 70,000 to 90,000 g/mol by gas permeation chromatography (GPC). PCL polydispersity was less than 2.0. Ibuprofen was obtained from Equilibrium LTDa, Brazil, presenting a molar mass of 206.27 g/mol and a density of 1.175 g/cm$^3$.

2.2. Blend preparation

Initially, PCL/Ibuprofen components were extruded to obtain a better mix of the polymer and drug. In this step the ibuprofen particles were dispersed in the PCL granules by mechanical mixing using a cylindrical blender for 30 min at 100 rpm with 10% Ibuprofen. The extrusion was carried out with a three temperature zone laboratory scale extruder with a single screw (LAB-14 - AXPlastic, Brazil). The length to diameter ratio (L/D) of the extruder was 20:1. The blend samples were produced using a constant screw speed of 50 rpm and a barrel zone temperature of 130 °C. This temperature was selected based on the melting point of PCL and ibuprofen, which are 60 °C and 76 °C, respectively. Therefore, it is ensured that both compounds be in the liquid state during the blend preparation. The temperature of 130 °C is not expected to change the bioactivity of ibuprofen.

2.3. Mold design and injection molding simulation

The CAD (computer aided design) models of the mesh and the mold were generated in SolidWorks®. Two distinct meshes were designed in the same mold cavity. The mesh 1 dimensions were set as 30 mm × 10 mm and 0.50 mm pore size while the mesh 2 dimensions were set as 30 mm × 10 mm and 0.80 mm pore size. Computation simulations of the injection molding process were used to validate the mold design. A computer aided engineering (CAE) system Moldflow® (Plastics Insight) evaluated the filling time, pressure and temperature for the mesh molding.

2.4. Manufacture of the aluminum prototype mold

The mold was manufactured by milling an aluminum block with a Roland MDX 540 machine. The computer-aided design (CAD) model of the mold was interfaced by a computer-aided manufacturing (CAM) process control system. The milling parameters were: a cut speed of 15,000 rpm and advancement speed of 0.01 mm/s. A rounded hard-metal tool of 500 μm diameter was used to prepare the mold (see Fig. 1).

2.5. Injection molding process

An Arburg 70S 250—70 injection molding machine was used for the injection process. The maximum clamping force of the machine is 250 kN. The screw in the plasticizing unit has a 15 mm diameter and a molding mass of 14.5 g. The mold temperature was kept at 30 °C with a Microquimica MBQ9920 temperature controller. Moldflow® CAE simulation was used to determine the time required to fill the entire mold (Fig. 2). The processing parameters selected were: an injection flow rate of 15 cm$^3$/s, a programmed maximum injection pressure of 1500 Bar, mold temperature of 30 °C, and temperature zones of 140, 180, 190 and 200 °C.

2.6. Microscopy analyses

The surface morphology of the mesh specimens were investigated with a Phillips XL30 scanning electron microscope (SEM). Before the analysis the specimens were coated with gold in a Bal-Tec Sputter Coater SCD005. Real dimensions of the pores from the stereomicroscope images were measured using the software ImageJ. The values reported here are the averages from three meshes for each type of sample, considering three pores per mesh. The standard deviations are also given.

2.7. Infrared spectroscopy and X ray diffraction

Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR) was performed in a Bruker spectrometer, model TENSOR 27, in the range of 4000 to 600 cm$^{-1}$ by accumulating 32 scans at a resolution of 4 cm$^{-1}$. The X-ray diffraction measurements were performed using a Philips PW1150 vertical diffractometer. The Cu–Ka nickel filtered radiation was detected in the range of 6–50°. The results of these analyses were used to control the physico-chemical properties of the meshes.

2.8. Mechanical tests

A Dynamic Mechanical Analyzer model DMA Q800 (TA instruments) with a tensile clamp was used for the mechanical tests. A force rate of 1 N/min from 0 to 18 N was applied for quasi-static tests. Creep-recover tests were performed applying 15 MPa of stress for 15 min and recovery was measured over a 30 min period. The creep-recover tests were performed at 37 °C. The mechanical tests were performed on three samples of PCL and PCL/Ibuprofen meshes for each pore dimension.

Fig. 1. CAD of (a) two meshes with different porosities and of (b) a single cavity mold for mesh injection molding.
2.9. Drug release test

PCL/IBP mesh specimens weighing 120 mg were immersed in 50 mL of phosphate buffer solution (pH = 7.4) (to maintain sink conditions). The samples were shaken horizontally in a Dubnoff bath (Quimis S.A, Brazil) at a rate of 60 rev/min to minimize the boundary effect and were maintained at a temperature of 37.0 ± 0.5 °C. The total receptor solution volume was replaced with fresh solutions at 48 h intervals. After suitable dilution with the buffer solution, the total drug release was obtained through a calibration curve using a UV-Vis spectrophotometry at $\lambda_{\text{max}}$ of 265 nm, on a Hitachi 2010 double-beam UV-visible spectrophotometer.

2.10. Cytotoxicity test by indirect contact

The cytotoxicity of the PCL and PCL/IBP prototype plates was evaluated by the indirect contact test using mesh eluates prepared by immersing the meshes in culture medium for three different lengths of time (1, 2 and 7 days). Cells from the immortalized cell line from L929 mouse fibroblasts were seeded and cultured for 24 h in multi-well culture plates containing the eluates to test whether the meshes released toxic products or not. Cell viability was measured after 24 h by the MTT viability assay. The absorbance measured by the MTT viability assay was normalized against the control.

2.11. Cytocompatibility tests for direct contact

In order to evaluate the cytocompatibility of the meshes, direct contact tests were performed by seeding human osteoblasts MG63 directly onto the PCL and PCL/IBP meshes samples. Due to the initial results of the cytotoxicity tests, in which the initial release of Ibuprofen triggered toxicity to the cells, it was decided to evaluate the direct cytocompatibility test using a PCL/IBP mesh after 48 h in sterile Phosphate Buffer Solution. Thus, cell viability after the initial high release could be evaluated. The viability of cells seeded on PCL and PCL/IBP meshes was evaluated using the MTT assay after 1, 3 and 7 days of culture.

2.12. Statistical analysis

All data presented are the mean ± standard deviation. The Student's t-test was used as test for statistical significance between two population and One-Way ANOVA was used between three populations. The Excel program and the OriginPro 7.5 software (OriginLab Corporation) were used. Statistical significance was considered at $p < 0.05$.

3. Results and discussion

PCL and PCL/ibuprofen meshes were prepared and characterized in terms of their physico-chemical, mechanical and biological properties. Microscopy images of PCL and PCL/IBP meshes are presented in Fig. 3. The dimensions reported in Table 1 are the averages of three meshes for each type, considering five pores in each mesh. The pore dimensions were not significantly different from the nominal size defined in the CAD project (0.5 mm and 0.8 mm).
0.8 mm). This result confirmed that injection molding using prototype mold, i.e. rapid tooling techniques can be used successfully to manufacture micro-porous meshes.

SEM micrographs of the PCL meshes with pores of 0.5 mm and 0.8 mm and PCL/IBP meshes with pores of 0.5 mm and 0.8 mm, are shown in Fig. 4 a, b and c, d, respectively. Both materials showed surfaces with irregularities, however, the PCL/IBP samples had greater roughness.

The SEM micrographs of PCL and PLC/IBP meshes (Fig. 5) enabled an evaluation of the mesh surfaces with a higher magnification (400X). The sample surfaces demonstrated similar features; however, IBP particles were found on the PCL/IBP surfaces (Fig. 5 c and d). EDS analysis clarified the chemical composition of the mesh and the particles (Fig. 5f). A higher concentration of oxygen was detected in the PCL matrix than in the particles, confirming the agglomeration of the drug on the surface of the sample.

X-ray analysis of PCL and PCL/IBP meshes with 0.5 mm and 0.8 mm pore sizes are shown in Fig. 6. Two peaks at 20 21.4 and 23.8 were identified in the diffractograms, which corresponded to the (110) and (200) planes of the orthorhombic crystalline structure of PCL [25,26]. The absence of IBP peaks in the spectra obtained for the PCL/IBP sample indicates the amorphization of the drug. The crystallinity of the PCL and PCL/IBP meshes are shown in Table 2. The values of crystallinity showed that PCL maintained its structural organization even after incorporation of the drug.

FTIR spectra for the ibuprofen (A), PCL (B and C) and PCL/Ibuprofen meshes (D-E) are shown in Fig. 7. The PCL meshes showed peaks at 2940, 2863, 1721, 1294, 1164, 1157 and 717 cm⁻¹ related to CH₂ asymmetric stretching, CH₂ symmetric stretching, C=O carbonyl stretching, C-O stretching in the crystalline phase, asymmetric C-O-C stretching, C-O stretching in the amorphous phase and CH₂ rocking, respectively (Fig. 7 b and c) [27]. The peaks

<table>
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<th>Table 1</th>
<th>Real dimensions of the pores measured on images from the microscope using ImageJ software.</th>
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<tr>
<td></td>
<td>Mesh Width</td>
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<td>---------</td>
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<tr>
<td>PCL 0.5</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>PCL 0.8</td>
<td>0.75 ± 0.02</td>
</tr>
<tr>
<td>PCL/IBP 0.5</td>
<td>0.44 ± 0.01</td>
</tr>
<tr>
<td>PCL/IBP 0.8</td>
<td>0.73 ± 0.02</td>
</tr>
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Fig. 4. SEM images of PCL meshes with 0.5 mm pores (A), PCL mesh with 0.8 mm pores (B), PCL/IBP meshes with 0.5 mm pores (C) and PCL/IBP mesh with 0.8 mm pores (D). Magnification of 20X.

Fig. 5. SEM images of PCL meshes with 0.5 mm pores (A), PCL mesh with 0.8 mm pores (B), PCL/IBP meshes with 0.5 mm pores (C) and PCL/IBP mesh with 0.8 mm pores (D). Magnification of 400X. EDS analyses of the IBP particles and PCL matrix (F).
present in the ibuprofen spectrum at 2955, 1721 and 1240 cm\(^{-1}\) are assigned to asymmetric CH\(_3\) stretching, C=O stretching and C-O stretching, respectively (Fig. 7a) [28], while the peaks at 668 and 580 cm\(^{-1}\) are related to the aromatic ring vibration in the ibuprofen structure. The FTIR spectra of the PCL/IBP meshes (Fig. 7d and e) gave peaks of PCL and IBP, which confirmed that the drug had been incorporated into the polymeric matrix.

Fig. 8a and b show representative stress versus strain curves obtained in the tensile test for the PCL and PCL/IBP meshes, respectively. The stiffness, maximum strength and elongation at the failure values for both materials are summarized in Table 3. No significant differences (p < 0.05) were measured between meshes of the different materials in stiffness, maximum strength and strain, for meshes with pores of 0.5 mm. For the meshes with pores of 0.8 mm, only the stiffness of the meshes showed significant

<table>
<thead>
<tr>
<th>Mesh</th>
<th>Crystallinity (%)</th>
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<tbody>
<tr>
<td>PCL 0.5 mm</td>
<td>52 ± 1</td>
</tr>
<tr>
<td>PCL 0.8 mm</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>PCL/IBP 0.5 mm 140 °C</td>
<td>52 ± 1</td>
</tr>
<tr>
<td>PCL/IBP 0.8 mm</td>
<td>53 ± 2</td>
</tr>
</tbody>
</table>

Fig. 6. Diffractograms of PCL and PCL/IBP meshes 0.5 mm (A) and 0.8 mm (B), respectively.

Fig. 7. Spectra of pure IBP (A), PCL meshes 0.5 mm (B) and 0.8 mm (C) and PCL/IBP meshes 0.5 mm (D) and 0.8 mm (E), respectively.
differences ($p < 0.05$) between the materials. The stiffness of PCL samples is significantly higher ($p < 0.05$) than the one of PCL/Ibuprofen. The average values for the stiffness and strain at failure of the PCL/Ibuprofen meshes were lower than for the PCL meshes probably due to the incorporation of the drug. PCL/Ibuprofen meshes were not affected by the difference of pore size.

No statistic significance ($p < 0.05$) was found in the values of stiffness, maximum strength and strain between the PCL/Ibuprofen meshes with porosity of 0.8 and 0.5 mm.

Creep–recovery tests were performed to evaluate the long-term mechanical behavior of the PCL and PCL/IbP meshes (Fig. 9). In terms of tensile strain, the PCL samples demonstrated better mechanical properties than PCL/IbP. Similarly, samples with lower porosity (0.5 mm) had better performance. The maximum recovery

![Stress versus strain curves for PCL (A) and PCL/IbP (B) meshes with porosities of 0.5 and 0.8 mm.](image1)

**Table 3**

<table>
<thead>
<tr>
<th>Mesh</th>
<th>Stiffness (MPa)</th>
<th>Strength (MPa)</th>
<th>Elongation at failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL 0.5 mm</td>
<td>22.1 ± 0.8</td>
<td>5.6 ± 0.7</td>
<td>330 ± 70</td>
</tr>
<tr>
<td>PCL 0.8 mm</td>
<td>24.9 ± 3.3</td>
<td>4.8 ± 0.1</td>
<td>90 ± 30</td>
</tr>
<tr>
<td>PCL/IbP 0.5 mm</td>
<td>16.6 ± 7.4</td>
<td>5.2 ± 0.2</td>
<td>170 ± 10</td>
</tr>
<tr>
<td>PCL/IbP 0.8 mm</td>
<td>14.1 ± 4.4</td>
<td>4.8 ± 0.1</td>
<td>80 ± 28</td>
</tr>
</tbody>
</table>

![Creep-recovery curves for PCL (A) and PCL/IbP (B) meshes with porosities of 0.5 and 0.8 mm.](image2)
observed was 60% of the strain for the PCL 0.5 mm samples, while for PCL 0.8 mm and PCL/IBP (0.5 and 0.8 mm) samples only 45% of maximum strain was reached. This result suggested the existence of a localized permanent viscous deformation in this type of mesh. In the first cycle, the curve slope was higher, indicating an important strain; however, the viscoelastic recovery was limited for PCL and PCL/IBP meshes and the deformation was not entirely recovered. The curves presented a less pronounced slope in the following cycles, indicating an elastic strain and reduced viscous deformations.

The release profiles of ibuprofen from PCL meshes with porosity of 0.5 and 0.8 mm are shown in Fig. 10a and b, respectively. The meshes showed a stable release confirming adequate process conditions. An initial fast release was observed in the first 3 days in which 20% of the ibuprofen was released. After 3 days the release was slower and controlled. This profile suggested that the release kinetics for ibuprofen in PCL meshes occurs initially from the elution of the drug onto the surface of the samples. Afterwards, the diffusion and erosion mechanisms contribute to the release of the drug. Higher concentrations of ibuprofen were detected from meshes with porosity of 0.5 mm (A), which may be associated to the higher surface area of these samples.

The cytotoxicity of the PCL and PCL/IBP meshes were evaluated by the indirect contact test. Eluates were prepared by immersing the meshes in culture medium for three different times (1, 2 and 7 days). Cells from the immortalized cell line from L929 mouse fibroblasts were seeded and cultured for 24 h in multi-well culture plates containing the eluates to test whether the meshes released toxic products or not. The MTT assay results are shown in Fig. 11. The PCL/IBP meshes showed a significant decrease in cell viability after 24 h, showing that the quantity of ibuprofen was toxic to the cells. It is interesting to note that the viability decreased by 80% in the first 24 h of the cell culture, but it remained almost constant from 2 to 7 days. On the contrary, the viability of cells cultured in the presence of the eluates obtained from PCL meshes is significantly higher and no significant decrease in the number of viable cells was detected after the 7-day-culture period. Furthermore, there are few and contrasting data regarding the toxicity of ibuprofen on cells and it is possible that the toxicity of the drug may have also been reinforced locally by a rapid delivery of a high dose from the drug delivery system. The amount of ibuprofen released into the medium in the first hours was around $8.0 \times 10^{-3}$ M. Considering a study by Cantón et al. [29] who found that cells tolerate concentrations of ibuprofen up to $10^{-3}$ M but show a cytotoxic response at concentrations of $10^{-4}$ M, it is clear that initial concentration of the drug released in this work had toxic effects on the cells. Other biomaterials, such as bone cements, have also been observed to trigger early toxicity. However, after a certain period of time the cells are able to grow and proliferate [30].

Direct contact tests evaluated the cytocompatibility of the PCL/IBP meshes by seeding human osteoblasts MG63 directly onto the PCL and PCL/IBP meshes. Based on previous cytotoxicity tests, the PCL/IBP meshes were immersed in sterile Phosphate Buffer Solution (PBS) for 48 h to eliminate the excess of IBP on their surfaces. Thus, cell viability after the initial release could be evaluated. The viability of cells seeded on PCL and PCL/IBP meshes was evaluated by the MTT assay after 1, 3 and 7 days of culture and the results are summarized in Fig. 12.

The results demonstrated the absence of cell toxicity after 1 and 3 days of direct contact, which confirmed that the initial toxic effect of IBP is due to the accumulation of drug on the surface of the sample. Since the cells were seeded on devices surfaces, and that adhesion is required for cell proliferation, it was assumed that cells adhered on device surface [31]. Similar results were found in previous studies, where a sustained release of ibuprofen could be attained without the production of a negative effect on the cell viability [32]. At the end of the first time period analyzed (1 day) both PCL and PCL/ibuprofen meshes presented cell viability higher...
than the control (p < 0.05). Moreover, the 0.8 mm porosity meshes loaded with the drug presented cell viability significantly higher than the other samples. During the 3 and 7-day-imersions the viability of the cells decreased uniformly reaching only 20% of viability on the last day of the experiment. The death of cells after 7 days is explained based on the degradation of PCL and the consequent release of acidic products that have toxic effect on cells adhering onto its surface. This effect was more pronounced in vitro due to the lower exchange of fluid and cell density close to the implant, in comparison to the in vivo environment [31,33].

4. Conclusion

This study investigated the preparation of PCL and PCL/IPB meshes for restorations of orbital floor fractures. Injection molding with prototyping mold and rapid tooling technique was employed. The pore size and drug content were observed to influence the mechanical behavior of the samples. The average values for the stiffness and strain at failure of PCL/Ibuprofen meshes were lower than for the PCL meshes probably due to the drug incorporation. Furthermore, the samples with porosity of 0.5 mm presented better mechanical properties. The drug delivery profile suggested that the release kinetics for ibuprofen in PCL meshes occurs initially by drug surface dissolution and after that by diffusion and erosion mechanisms. The cytotoxicity tests performed on PCL and PCL/Ibuprofen meshes indicated that initial concentration released triggered toxicity to the cells. However, after immersing the sample in PBS for 2 days and testing the samples by the direct contact tests the PCL/IPB meshes interacted well with the cells seeded on their surfaces, as indicated by the cytocompatibility tests.

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