

# Hyperspectral chemical imaging reveals spatially varied degradation of polycarbonate urethane (PCU) biomaterials

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1           **Hyperspectral chemical imaging reveals spatially varied degradation of**  
2                           **polycarbonate urethane (PCU) biomaterials**

3  
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14

1 **Abstract**

2 Hyperspectral chemical imaging (HCI) is an emerging technique which combines  
3 spectroscopy with imaging. Unlike traditional point spectroscopy, which is used in the  
4 majority of polymer biomaterial degradation studies, HCI enables the acquisition of spatially  
5 localised spectra across the surface of a material in an objective manner. Here, we  
6 demonstrate that attenuated total reflectance Fourier transform infra-red (ATR-FTIR) HCI  
7 reveals spatial variation in the degradation of implantable polycarbonate urethane (PCU)  
8 biomaterials. It is also shown that HCI can detect possible defects in biomaterial formulation  
9 or specimen production; these spatially resolved images reveal regional or scattered spatial  
10 heterogeneity. Further, we demonstrate a map sampling method, which can be used in  
11 time-sensitive scenarios, allowing for the investigation of degradation across a larger  
12 component or component area. Unlike imaging, mapping does not produce a contiguous  
13 image, yet grants an insight into the spatial heterogeneity of the biomaterial across a larger  
14 area. These novel applications of HCI demonstrate its ability to assist in the detection of  
15 defective manufacturing components and lead to a deeper understanding of how a  
16 biomaterial's chemical structure changes due to implantation.

17 **Keywords:** Biomaterial characterisation, biostability, hyperspectral chemical imaging, *in vivo*  
18 degradation, polycarbonate urethane.

19

20

## 1 **1. Introduction**

2 A biomaterial is a material intended to interface with biological systems to evaluate, treat,  
3 augment or replace any tissue, organ or function of the body [1]. The human body is an  
4 aggressive environment for biomaterials [2] and may have an adverse effect on the  
5 performance of these materials [3]. To gain an understanding of the failure mechanisms  
6 associated with the *in vivo* performance of an implanted device (and its biomaterial  
7 components), it is important to examine explanted (retrieved) devices [4]. Mechanical wear  
8 and damage characterisation are important in retrieval analysis studies, however,  
9 investigations relating to changes to chemical structure due to the biological environment  
10 are equally necessary [4]. Long-term implantable polymers are an important class of  
11 biomaterials, used in a variety of biomedical applications [5]. In particular, the polyurethane  
12 (PU) group, and more specifically polycarbonate urethanes (PCU), are used in vascular  
13 catheters [6,7] and orthopaedic [8–11] applications. Though PCU has been shown to be  
14 more biostable than polyether urethanes (PEU) [12,13], another polymer in the PU group  
15 used in cardiovascular applications [14], PCU components of explanted orthopaedic  
16 implants have been reported to degrade in the human body due to oxidation [9,15]. This  
17 oxidative degradation results in the cleavage of chemical bonds in a polymer [16] and has  
18 been shown to adversely affect the surface chemical structure of the PCU spacer of the  
19 Dynesys spinal stabilisation device (Zimmer, Warsaw, Indiana, USA) [9,15,17,18]. To  
20 replicate and understand the effect of *in vivo* oxidation on chemical structure, an  
21 accelerated *in vitro* oxidation method to degrade PCU biomaterials has been reported  
22 [8,12,19]. This process produces hydroxyl radicals from the H<sub>2</sub>O<sub>2</sub>/CoCl<sub>2</sub> solution and is  
23 reported to be an appropriate model of the *in vivo* processes which produce oxygen radicals

1 at the polymer/cell interface [20]. By using this *in vitro* degradation method, it was shown  
2 that oxidative degradation also affects the viscoelastic properties of the BDyn spinal  
3 stabilisation device (S14 Implants, Pessac, France), and its components, at specific  
4 frequencies [8].

5 Fourier transform infra-red (FTIR) spectroscopy is one of the three recommended  
6 techniques to quantify the chemical structure of polymers according to the ISO standard  
7 10993-18, in addition to nuclear magnetic resonance (NMR) spectroscopy and mass  
8 spectroscopy (MS). Attenuated total reflectance Fourier transform infra-red (ATR-FTIR)  
9 spectroscopy has been used to understand and characterise the chemical structure changes  
10 of films [12,19,21–23], tubing [21], dumbbell or cylindrical specimen shapes [24–28],  
11 pacemaker or defibrillator leads [14,29] and orthopaedic devices [8,9,15,17,18]. A major  
12 limitation of standard ATR-FTIR, as conventionally applied, is that spectra are usually  
13 acquired from a single point of a sample. This risks missing critical information of how, and  
14 the extent to which, degradation varies across an explant spatially, as well as potential  
15 operator bias in the selection of an acquisition point on an explanted component. To  
16 overcome these potential risks, the acquisition of spatially resolved chemical data is  
17 desirable. Hyperspectral chemical imaging (HCI) is an emerging technique which combines  
18 spectroscopy with imaging, enabling the acquisition of spatially localised spectra across the  
19 surface of a material [30,31]. The feasibility of mid-infrared HCI has been demonstrated for  
20 high-throughput, non-destructive monitoring of biochemical processes directly at the  
21 cellular level [32], evaluation of the molecular state of polymer matrices for drug release  
22 [33] and protein adsorption on monolayers [34]. ATR-FTIR HCI enables the acquisition of  
23 spectra from the surface of samples that would otherwise be too thick for conventional mid-

1 IR spectroscopy. A tutorial on the acquisition of hyperspectral chemical images and  
2 subsequent computational analysis (through both Matlab and R) has been previously  
3 presented [35]. However, to the best of the authors' knowledge, the potential of this  
4 technique for evaluation of surface variations in the degradation of long-term implantable  
5 biomaterials has not been evaluated. Here, we demonstrate that ATR-FTIR HCl reveals  
6 spatial variations in the degradation of polymer biomaterials. Two long-term implantable  
7 PCUs (Bionate II 80A and ChronoFlex C 80A) were degraded by two different *in vitro*  
8 oxidation methods. Comparisons, based on HCl data, were made between the untreated  
9 specimens and specimens degraded through *in vitro* oxidation. Further, this study  
10 investigated and reports variations of the *in vivo* degradation of an explanted biomaterial  
11 component from an orthopaedic device using HCl.

## 12 **2. Materials and methods**

### 13 **2.1 Biomaterials**

14 Bionate II 80A (DSM, Heerlen, Netherlands) and ChronoFlex C 80A (AdvanSource  
15 Biomaterials, Wilmington, MA, USA) are commercially available long term implantable  
16 polycarbonate urethane (PCU) biomaterials. These were gathered in pellet form and  
17 injection moulded into 150 mm x 150 mm sized plaques [36] with a thickness of 3 mm  $\pm$  0.2  
18 mm according to the individual manufacturers' recommendations. As per implantation  
19 protocols, the plaques were sterilised by Ethylene Oxide (EtO) (Steriservices, Bernay,  
20 France). This process involves three cycles with an EtO concentration of 600 mg/L for 4  
21 hours at 43 °C. No separate aeration was implemented as aeration was performed in the  
22 chamber. The plaques were then cut into the defined ASTM D1708 specimen shape [37] by  
23 using a die cutter (Wallace Instruments, Cambridge, UK) on a Wallace Hand Operated

1 Specimen Cutting Press (Wallace Instruments, Cambridge, UK). From each plaque, 23  
2 specimens were cut. Once a plaque was cut, the specimens were numbered and a random  
3 order generator (Excel 2010, Microsoft, Redmond, Washington, USA) was used to separate  
4 the specimens into untreated (n=6), 3% H<sub>2</sub>O<sub>2</sub> (n=6) and 20% H<sub>2</sub>O<sub>2</sub> / 0.1M (n=6) groups.

## 5 **2.2 *In vitro* degradation of biomaterials**

6 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Fisher Scientific, Loughborough, United Kingdom) and 0.1M  
7 cobalt (II) chloride hexahydrate (CoCl<sub>2</sub>.6H<sub>2</sub>O) (Sigma Aldrich, Dorset, United Kingdom) were  
8 used in the preparation of the oxidative degradation solutions. The 30% H<sub>2</sub>O<sub>2</sub> was diluted to  
9 the appropriate 3% H<sub>2</sub>O<sub>2</sub> and 20% H<sub>2</sub>O<sub>2</sub> concentration levels using deionized water [13].

10 The ISO 10993-13 method was followed to degrade Bionate II 80A and ChronoFlex C 80A  
11 with 3% H<sub>2</sub>O<sub>2</sub>. Oxidative degradation of the biomaterials was performed at 37°C ± 1°C in a  
12 water bath (JB5 Water Bath, Grant Instruments, Cambridgeshire, United Kingdom). The  
13 solution was changed every seven days and the degradation period was 52 weeks. The  
14 solution did not include any metal ions to catalyse the decomposition of the hydrogen  
15 peroxide [24]. After the degradation period, the specimens were rinsed with deionised  
16 water and dried under vacuum for 48 hours at room temperature.

17 The commonly used accelerated oxidation method [8,12,19] was also used to degrade the  
18 PCU biomaterials with 20% H<sub>2</sub>O<sub>2</sub>/0.1M CoCl<sub>2</sub>.6H<sub>2</sub>O. To maintain a relatively constant  
19 concentration of radicals [19], the H<sub>2</sub>O<sub>2</sub>/CoCl<sub>2</sub>.6H<sub>2</sub>O solution was changed every three days  
20 while the degradation period lasted 24 days. Similar to the ISO 10993 method, accelerated  
21 oxidative degradation was performed at 37°C ± 1°C in a water bath (JBN18 Water Bath,  
22 Grant Instruments, Cambridgeshire, United Kingdom). After the degradation period, the

1 specimens were rinsed with deionised water and were dried under vacuum for 48 hours at  
2 room temperature.

### 3 **2.3 BDyn Posterior Dynamic Stabilisation device**

4 The BDyn Posterior Dynamic Stabilisation (PDS) device (S14 Implants, Pessac, France) is a  
5 bilateral system designed to preserve intersegmental range of motion, reduce intradiscal  
6 pressure and alleviate loading of the facet joints [11]. It comprises of a polycarbonate  
7 urethane ring (Bionate II 80A, DSM, Heerlen, The Netherlands) and a silicone cushion (MED-  
8 4770, NuSil Technology LLC, Carpinteria, CA, USA), a mobile titanium alloy rod, a fixed  
9 titanium alloy rod and is fixed to the vertebrae by titanium alloy pedicle screws [11]. The  
10 BDyn explant was sent to S14 Implants (Pessac, France) after complications with the device.  
11 The titanium alloy housing was carefully cut along a laser weld to expose the PCU and  
12 silicone components and the components were sterilised. Patient details were  
13 unobtainable, however, the estimated implantation time (based on distribution of implants  
14 to retrieval surgery date) was approximately six months.

### 15 **2.4 Hyperspectral Chemical Imaging**

16 A Thermo Scientific™ Nicolet™ iN™10 Infrared Microscope (10× magnification, Mercury-  
17 Cadmium-Tellurium (MCT) detector, working range 4000-675  $\text{cm}^{-1}$  with a 2  $\text{cm}^{-1}$  spectral  
18 resolution) was used in combination with a slide-on Germanium ATR Micro Tip to collect  
19 ATR-FTIR hyperspectral chemical images. The system was nitrogen gas purged for an hour  
20 before use and the purge was maintained during use. The detector was a liquid nitrogen  
21 cooled and allowed to equilibrate for an hour before use. This was also maintained during  
22 acquisition. Specimens were attached to glass slides and inserted into the instrument. A  
23 white light mosaic image was collected to aid with hyperspectral collection.



### 1 **2.4.1 Imaging of *in vitro* degraded biomaterials**

2 A detector aperture size of 200  $\mu\text{m}$  was selected, and the resultant spatial resolution of the  
3 ATR Micro Tip using the single point detector was 50  $\mu\text{m}$ . After selecting a region of interest  
4 in the centre of each biomaterial sample, grids of data points were selected such that the  
5 distance between each pixel was 50  $\mu\text{m}$ , in order to generate spatially contiguous images.  
6 Pixel acquisition time was  $\sim 11.2$  seconds and images were 1296 pixels in size resulting in a  
7 total acquisition time of  $\sim 4$  hours (typically the collective acquisition times of  $x$  spectra will  
8 be approximately  $x$  times longer than the collection of a single spectrum).

### 9 **2.4.2 Imaging of *in vivo* degraded explant**

10 An aperture size of 150  $\mu\text{m}$  was selected, and the resultant spatial resolution of the ATR  
11 Micro Tip using the single point detector was 37.5  $\mu\text{m}$ . After selecting a region of interest in  
12 the explant specimen, a grid of data points was selected such that the distance between  
13 each pixel was 100  $\mu\text{m}$ , in order to generate a spatially resolved (non-contiguous)  
14 hyperspectral chemical map.

### 15 **2.4.3 Raman mapping**

16 In order to independently corroborate the ATR-FTIR results, identical areas of Bionate II 80A  
17 materials were analysed using both ATR-FTIR and Raman spectroscopy mapping. These  
18 materials were from the same experimental batch as the *in vitro* studies described above in  
19 section 2.2 but were examined 6 months after the original experimental protocol and after  
20 subsequent mechanical testing of the samples.

21 Raman maps were acquired using an inVia Micro-Raman confocal spectroscopy system  
22 (Renishaw, Wotton-under-Edge, Gloucestershire, UK) with a 10x 0.25NA objective lens, 785

1 nm edge laser (laser power was 306 mW at source, and was set to 1% power for sample  
2 measurements) and an 600 line  $\text{mm}^{-1}$  grating without pinhole. Spectra were calibrated to a  
3 silicon shift at  $520 \text{ cm}^{-1}$ . The detector used was a NIR enhanced Deep Depletion CCD array  
4 ( $1024 \times 256$  pixels) which was Peltier cooled to  $-70^\circ\text{C}$ . The spectral range was  $3035.3 \text{ cm}^{-1}$  -  
5  $1089.1 \text{ cm}^{-1}$  with a mean spectral resolution of  $1.9231 \text{ cm}^{-1}$  over 1013 measured spectral  
6 bands. As contiguous Raman imaging over a similar area to the ATR images was not feasible  
7 within a reasonable timeframe, areas were mapped rather than imaged (using a step size of  
8  $15 \mu\text{m}$ ). The laser footprint was rectangular/oval in shape, with the longer dimension  
9 mapping to the vertical direction of the generated Raman map. As the step size was equal in  
10 X and Y directions, this resulted in a vertically directed elongated appearance in features of  
11 the Raman images.

12 ATR-FTIR imaging parameters were as described in section 2.4.1.

## 13 **2.5 Pre-processing**

14 ATR-FTIR spectra were pre-processed as described below. The spectral range was cut to  
15  $2000 \text{ cm}^{-1}$  -  $675 \text{ cm}^{-1}$ . The spectra were then scaled to a stable aromatic C-H out of plane  
16 bending peak at  $819 \text{ cm}^{-1}$  [9]. Linear baseline correction was subsequently performed and  
17 followed by a baseline shift of one arbitrary unit. A peak at  $\sim 1591\text{-}1596 \text{ cm}^{-1}$  indicative of  
18 C=C bond stretching of an aromatic ring of the hard segment [12,21,23,38] has been  
19 reported to remain unchanged in PCU oxidation [39], and has therefore been used as  
20 internal reference peak in the literature. However such an approach is not suitable in the  
21 present instance as a neighbouring peak overlaps the C=C stretching peak and consistency  
22 of its intensity between spectra cannot be assumed. In other studies PCU spectra have been

1 normalised to an aromatic internal reference peak at  $508\text{ cm}^{-1}$  [4], however this was outside  
2 of the measured range.

3 Internal normalisation was not possible in the case of the *in vivo* specimen image due to the  
4 disappearance of the  $819\text{ cm}^{-1}$  peak in highly degraded regions. For this reason, SNV  
5 normalisation (spectrum-wise mean subtraction with spectrum-wise division by standard  
6 deviation) was used in the case of the *in vivo* image.

7 No pre-processing was applied to the Raman spectra.

## 8 **2.6 Data analysis**

9 The mean spectrum was calculated for each image and randomised sample spectra were  
10 inspected. Randomisation was achieved through the “rand” function of Matlab. Histograms  
11 were produced at wavenumbers of interest in order to assess the shape of absorbance  
12 distributions. ATR images at particular wavenumbers of interest were inspected.

13 Peak ratio images were also generated. The ratio between peaks reported to be indicative  
14 of degradation ( $1650\text{ cm}^{-1}$  (growing): aromatic amine and  $1248\text{ cm}^{-1}$  (shrinking): carbonate  
15 [12,17,21]) were calculated and resultant dimensionless ratio value images were generated.  
16 Trends in these images were compared to the trends of the single wavenumber images.

17 The “parula” (blue → green → yellow) colour scale was used for the image generation of all  
18 *in vitro* images as this colour scale ranges from dark to bright incrementally. This is an  
19 important consideration as colour scales which do not possess this property can result in  
20 human error when arbitrary dramatic colour changes cause the observer to believe a  
21 greater difference exists than is actually the case. The “hot” colour scale was used in all

1 overlaid *in vivo* maps as this colour scale increments from dark to bright but also contrasts  
2 well with the white-light image onto which it has been overlaid.

3 Multivariate analysis was conducted in relation to *in vitro* specimens only. Reference spectra  
4 were chosen to represent both degraded and undegraded materials. The mean control  
5 spectrum (B0) was chosen as an undegraded reference spectrum, while the mean spectrum  
6 of the material exposed to the harshest oxidation technique (B20) was chosen as a  
7 degraded reference spectrum. The undegraded reference spectrum was then subtracted  
8 from the degraded reference spectrum to produce a difference spectrum which highlights  
9 features associated with degradation. Each HCI (B0, B3 and B20 *in vitro* Bionate II 80A  
10 images) was then projected along the difference spectrum, by multiplying the HCI spectra by  
11 the difference spectrum, and converting each pixel-spectrum to a single index  
12 representative of the level of degradation. A histogram was produced to assess the  
13 distribution of these indices and to select threshold points for separation of groups of pixels  
14 into classes according to degradation level. Finally, the degradation classes were visualised  
15 as images and the mean spectrum of each class was inspected. The time taken to process  
16 the data (including initial loading and image generation) was 28.5seconds, on Windows 10  
17 running Matlab 2017b.

## 18 **2.7 Statistical analysis**

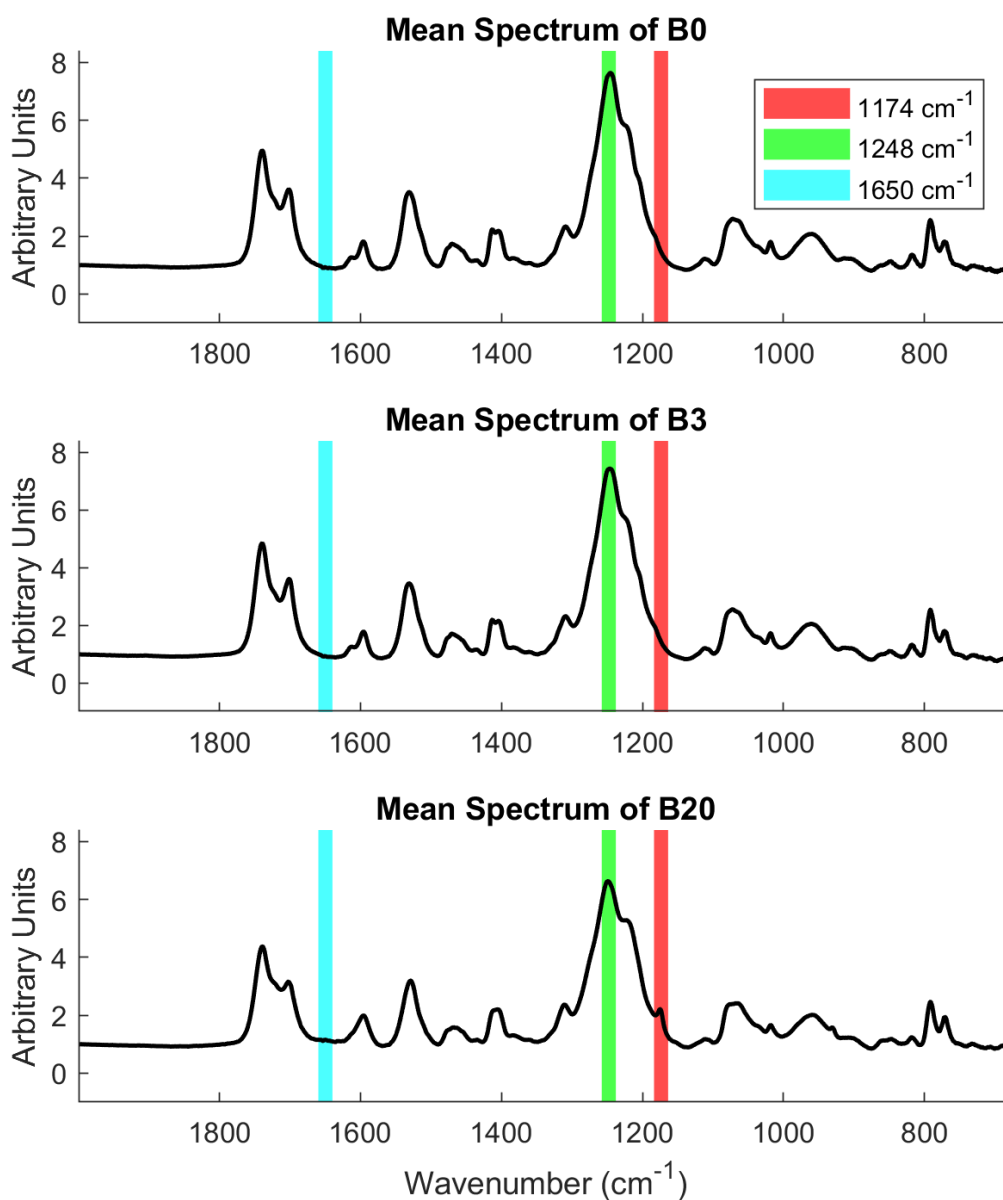
19 Each HCI comprised 1296 spectra. Descriptive statistics, i.e. the sample means and standard  
20 deviations of the intensities of the peaks of interest ( $1174\text{ cm}^{-1}$ ,  $1248\text{ cm}^{-1}$  and  $1650\text{ cm}^{-1}$ )  
21 were calculated from each image.

22

## 1 **3. Results**

### 2 **3.1 Univariate analysis of Bionate II (*in vitro* degradation)**

3 The mean spectra of the hyperspectral chemical images of control (B0), 3% H<sub>2</sub>O<sub>2</sub> (B3) and  
4 20% H<sub>2</sub>O<sub>2</sub>/0.1M CoCl<sub>2</sub>·6H<sub>2</sub>O (B20) treatments for Bionate II 80A (DSM, Heerlen, Netherlands)  
5 were calculated (Fig. 1). As reported in the literature [12,17,21] 1174 cm<sup>-1</sup> is indicative of C-  
6 C cross-linking, 1248 cm<sup>-1</sup> is indicative of C-O-C carbonate bonds, and 1650 cm<sup>-1</sup> is indicative  
7 of aromatic amines (Supplementary Table 1). It was found that little to no C-C cross-linking  
8 peak exists at 1174 cm<sup>-1</sup> in the mean spectra of the B0 and B3 specimens, while a small yet  
9 notable peak emerges in the B20 specimen mean spectrum. Further, it is evident that the  
10 carbonate peak at 1248 cm<sup>-1</sup> decreased in the B20 specimen relative to the B0 and B3  
11 specimens, suggesting degradation of the carbonate soft segment. Finally, no aromatic  
12 amine peak or band was detected at 1650 cm<sup>-1</sup> in the mean spectra of B0 and B3, however,  
13 a subtle band is visible in the B20 mean spectrum.



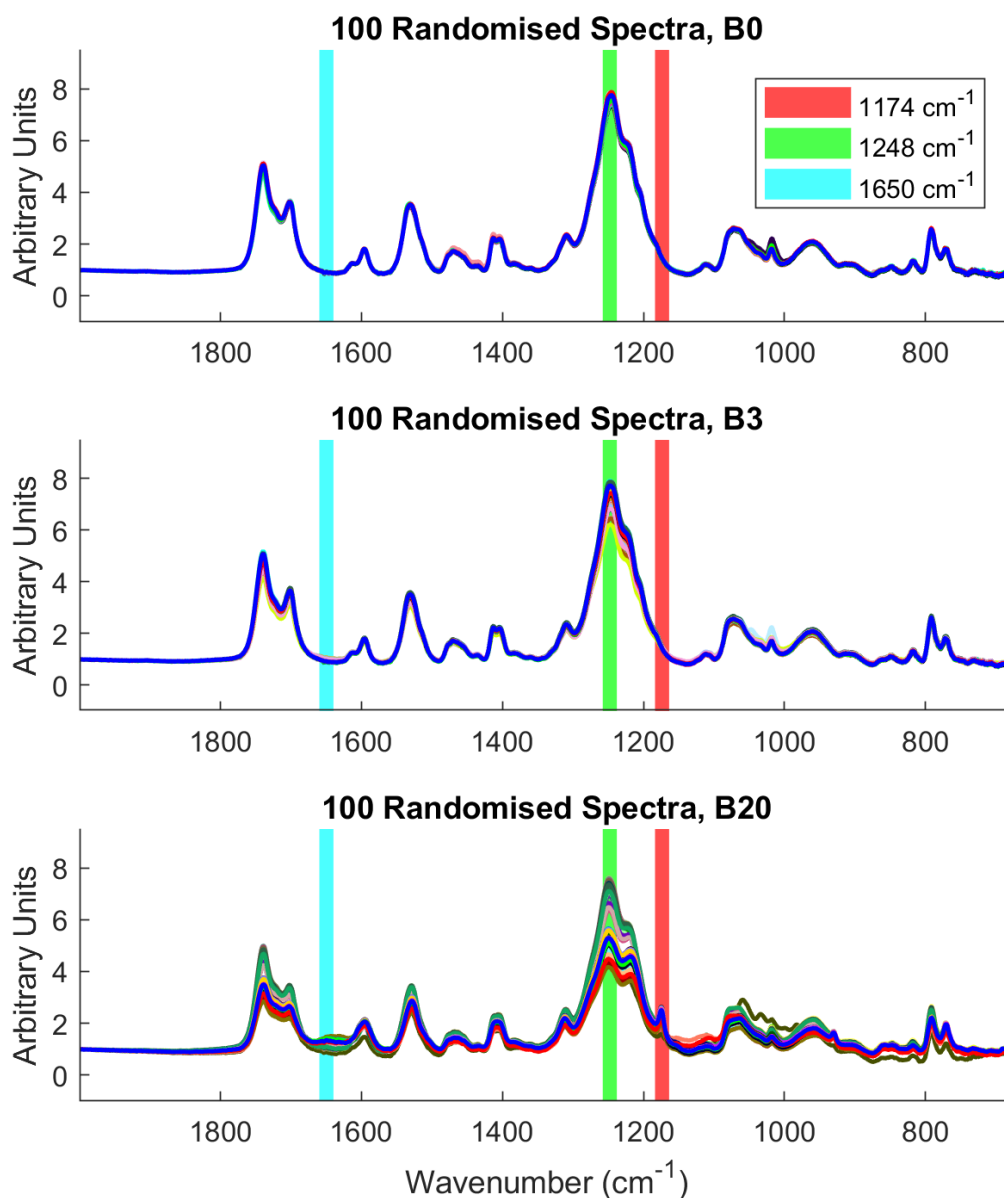
1

2 **Figure 1. Image mean spectra of B0 (top), B3 (middle) and B20 (bottom) for Bionate II 80A. Colour bars have**  
 3 **been added to indicate the spectral ranges associated with polycarbonate urethane degradation (1174 cm<sup>-1</sup>**  
 4 **[range: 1183 cm<sup>-1</sup> to 1160 cm<sup>-1</sup>]: C-C cross-linking, 1248 cm<sup>-1</sup> [range: 1260 cm<sup>-1</sup> to 1235 cm<sup>-1</sup>]: C-O-C**  
 5 **carbonate, and 1650 cm<sup>-1</sup> [range: 1662 cm<sup>-1</sup> to 1630 cm<sup>-1</sup>]: aromatic amine) (Supplementary Table 1).**

6

7 To inspect whether the mean spectrum is an accurate representation of each specimen, 100  
 8 spectra were randomly selected from each hyperspectral chemical image (Fig. 2). While B0

1 and B3 show little variability between spectra (and their corresponding mean spectra shown  
2 in Fig. 1), this is not the case for the B20 spectra which exhibit considerable variability within  
3 the sample, particularly in the case of the aromatic amine peak at  $1650\text{ cm}^{-1}$  and the  
4 carbonate peak at  $1248\text{ cm}^{-1}$ .

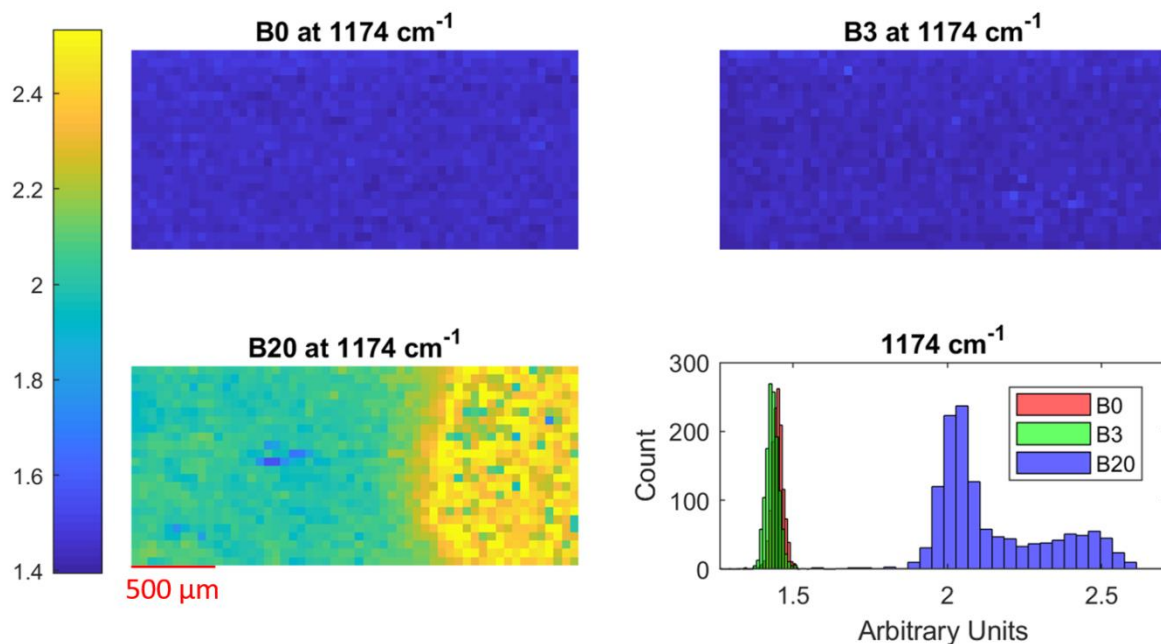


5  
6 **Figure 2. 100 randomised spectra from hyperspectral chemical image of B0 (top), B3 (middle) and B20**  
7 **(bottom) for Bionate II 80A. Colour bars have been added to indicate the spectral ranges associated with**  
8 **polycarbonate urethane degradation ( $1174\text{ cm}^{-1}$  [range:  $1183\text{ cm}^{-1}$  to  $1160\text{ cm}^{-1}$ ]: C-C cross-linking,  $1248\text{ cm}^{-1}$**

1 [range: 1260 cm<sup>-1</sup> to 1235 cm<sup>-1</sup>): C-O-C carbonate, and 1650 cm<sup>-1</sup> [range: 1662 cm<sup>-1</sup> to 1630 cm<sup>-1</sup>): aromatic  
2 amine).

3 To further investigate heterogeneities in each sample, single wavenumber images were  
4 generated at the wavenumbers reported to be related to degradation [12,24], accompanied  
5 by corresponding histograms (Fig. 3, Supplementary Fig. 1 and Supplementary Fig. 2). From  
6 visual inspection, no C-C cross-linking (at 1174 cm<sup>-1</sup>) was detected in the B0 and B3 images  
7 (Fig. 3). However, the entire imaged surface of the B20 specimen has experienced C-C cross-  
8 linking. Interestingly, the degree to which C-C cross-linking has occurred is highly variable. In  
9 the case of B0 and B3, the histogram (Fig. 3, bottom right) shows a mono-modal distribution  
10 in relation to 1174 cm<sup>-1</sup>. These distributions appear to substantially overlap, with similar  
11 means (1.45 and 1.43) and standard deviations (0.02 and 0.02) for B0 and B3 respectively,  
12 while the B20 sample had a larger mean (2.15) and standard deviation (0.19). A bi-modal  
13 distribution at 1174 cm<sup>-1</sup> can be observed in the case of B20, suggesting that there are at  
14 least two degradation populations in the B20 specimen. The corresponding B20 image (Fig.  
15 3, bottom left) reveals the spatial arrangement of these degradation populations.





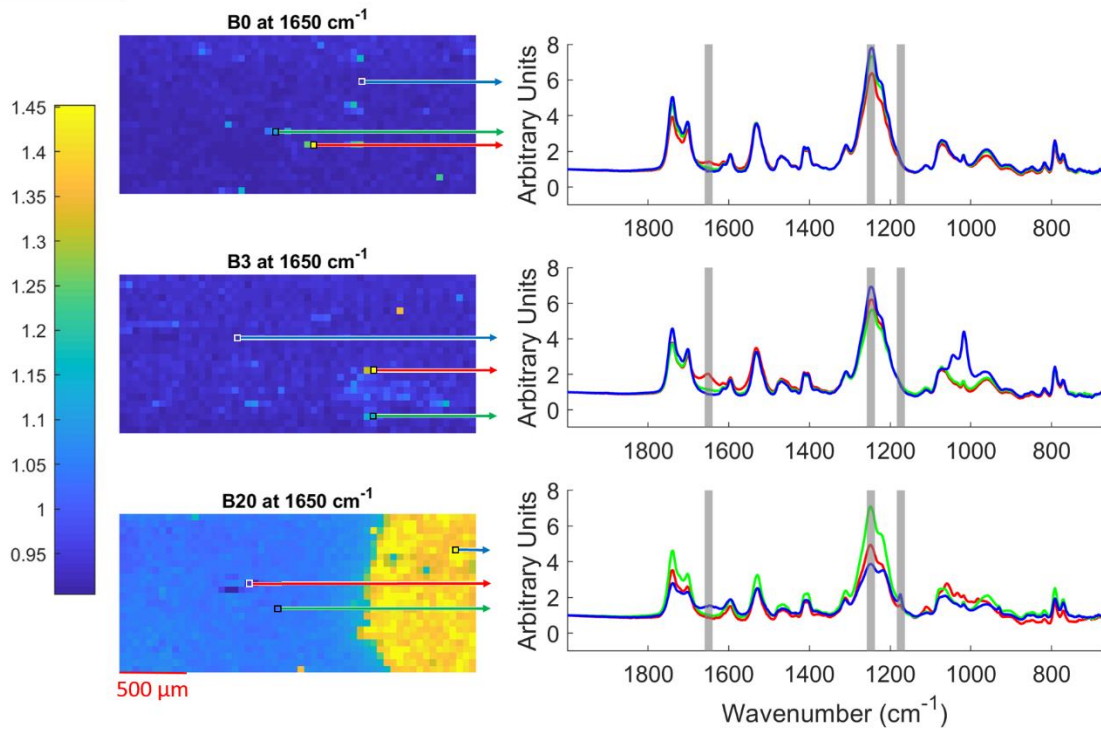
1

2 **Figure 3. Images of B0 (top left), B3 (top right) and B20 (bottom left), with accompanying histogram (bottom**  
 3 **right) depicting relative absorbance at 1174 cm<sup>-1</sup>. Image colour scale from 1<sup>st</sup> to 99<sup>th</sup> percentile.**

4 The distribution of the 1248 cm<sup>-1</sup> carbonate peak varied across the surface of all samples  
 5 (Supplementary Fig. 1). The histogram demonstrates a mono-modal distribution in the case  
 6 of both B0 and B3, which have similar means (7.58 and 7.42) and similar standard deviations  
 7 (0.18 and 0.20), while B20 has in this case a smaller mean (6.61) and larger standard  
 8 deviation (1.08). Once again, in the case of B20, a bi-modal distribution is visible in the  
 9 histogram (Supplementary Fig. 1, bottom right) which is spatially resolved in the  
 10 accompanying image (Supplementary Fig. 1, bottom left). While, from the histogram, it  
 11 appears that there is a mono-modal 1248 cm<sup>-1</sup> distribution in the case of B0 and B3, several  
 12 pixels of their corresponding 1248 cm<sup>-1</sup> images (Supplementary Fig. 1, top left and right  
 13 respectively) appear to be of lower intensity than the majority, suggesting small scattered  
 14 regions of carbonate soft segment degradation. These regions are not clearly distinguishable  
 15 in the histogram due to their scarcity. Equivalently, left and central regions of the B20 1248

1  $\text{cm}^{-1}$  image (Supplementary Fig. 1, bottom left) comprise pixels of considerably higher  
2 intensity (brighter pixels) than the image mean, suggesting a lack of carbonate degradation  
3 in this region. An emerging peak at  $1650 \text{ cm}^{-1}$ , associated with the formation of aromatic  
4 amines, is generally indicative of hard segment degradation [12,24] (Supplementary Fig. 2).  
5 As before, upon inspection of the histogram (Supplementary Fig. 2, bottom right), B0 and B3  
6 can be described as comprising a mono-modal distribution (again with similar means (0.93  
7 and 0.94) and standard deviations (0.03 and 0.04)) and B20 can be seen to comprise a bi-  
8 modal distribution with a mean of 1.15 and standard deviation of 0.16 (Supplementary Fig.  
9 2). Similar to the  $1248 \text{ cm}^{-1}$  images, the  $1650 \text{ cm}^{-1}$  images are not homogeneous, with  
10 apparent outlier pixels visible in all three specimen images at the same positions as those of  
11 the  $1248 \text{ cm}^{-1}$  images.

12 To further investigate the apparent outlier pixels of the single wavenumber images,  
13 individual pixels were selected from the  $1650 \text{ cm}^{-1}$  images and their associated spectra were  
14 visualised to assess their chemical differences (Fig. 4).



1  
2 **Figure 4. Spectra of pixels selected from images of relative absorbance at 1650 cm<sup>-1</sup> for B0, B3 and B20. All**  
3 **images scaled equally (height and width). Image colour scale from 1<sup>st</sup> to 99<sup>th</sup> percentile.**

4 It can be seen that bright pixels indicate the presence of a greater 1650 cm<sup>-1</sup> absorbance  
5 intensity in the associated spectrum, while the darker pixels indicate a lower absorbance  
6 intensity at the 1650 cm<sup>-1</sup> position. Observation of the selected spectra indicates they are  
7 not truly outliers (e.g. due to errors in detection), but rather represent highly localised  
8 specimen regions with different chemical features. Cell-biomaterial interactions in these  
9 regions could vary significantly from the un-degraded surface regions, resulting in potential  
10 complications with an implant. For example, regions of degradation could act as sites for  
11 protein adhesion, leading to issues such as biofilm formation. Similar spatial features are  
12 evident when comparing the 1650 cm<sup>-1</sup> images to the dimensionless peak ratio images  
13 (Supplementary Figures 3 and 4). In these peak ratio images, the same pixel intensity trends  
14 were observed regardless of normalisation.

1 Raman hyperspectral chemical maps were compared with ATR-FTIR HCIs as a means of  
2 independent corroboration of the results. Supplementary Figures 5 (B0) and 6 (B20) present  
3 mean Raman images, and ATR-FTIR images at  $1650\text{ cm}^{-1}$ . Changes in measured fluorescence  
4 appeared to be a major cause of the differences observed between Raman pixels. It should  
5 also be noted that features which are IR active are not equivalently active in the case of  
6 Raman spectroscopy, leading to changes in relative intensity of pixel features between  
7 modalities. Furthermore, the Raman laser footprint is considerably smaller than the ATR-  
8 FTIR detection area. Therefore smaller features could be resolved by this technique.  
9 Nevertheless, similar pixel patterns, indicating spatially varying degradation, were detected  
10 with both modalities, thus corroborating the observed results.

11

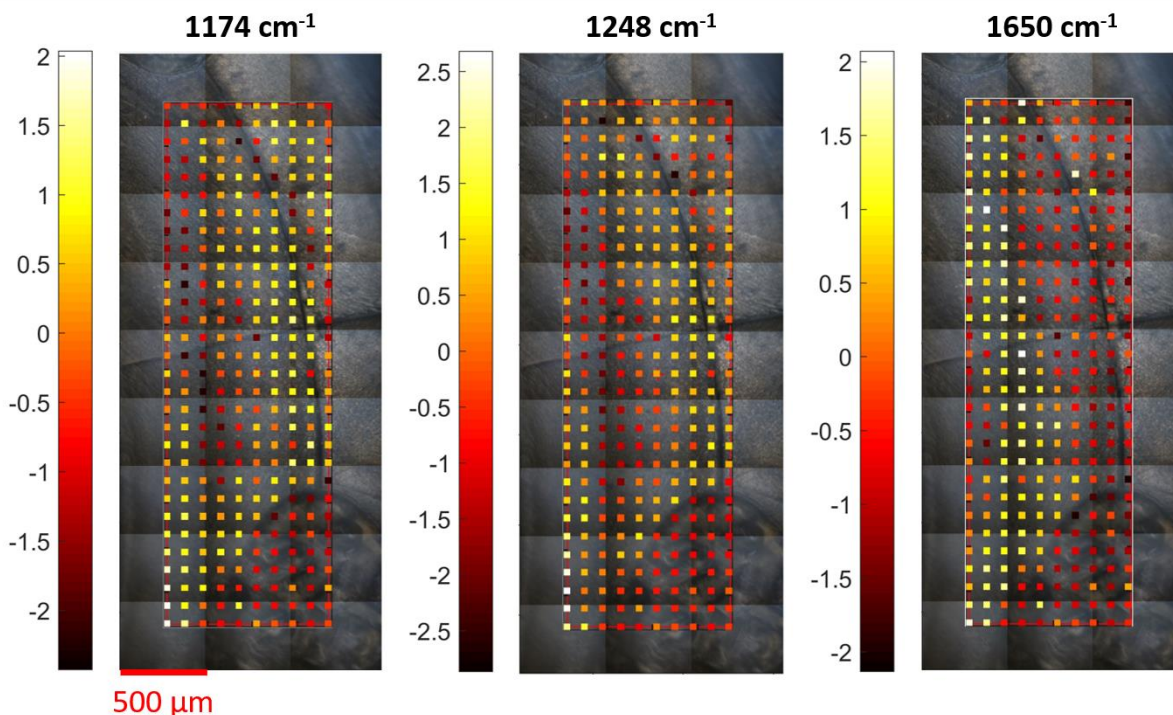
### 12 **3.2 Univariate analysis of ChronoFlex C (*in vitro* degradation)**

13 Similar observations were made in relation to other PCU biomaterials, specifically  
14 ChronoFlex C 80A (AdvanSource Biomaterials, Wilmington, MA, USA). The HCI mean  
15 spectrum of a ChronoFlex C 80A specimen subjected to the B20 oxidation method, appears  
16 to show a subtle aromatic amine peak at  $1650\text{ cm}^{-1}$  as well as a C-C cross-linking peak at  
17  $1174\text{ cm}^{-1}$  (Supplementary Fig. 7, top). The peak at  $1248\text{ cm}^{-1}$  may have reduced in relative  
18 intensity, however, this is difficult to determine from a single mean spectrum. When 100  
19 randomised spectra were assessed (Supplementary Fig. 7, bottom), it was found that the  
20  $1248\text{ cm}^{-1}$  intensity varied considerably. To assess the pixel intensity distributions for the  
21 wavenumbers of interest, single wavenumber histograms (at  $1174\text{ cm}^{-1}$ ,  $1248\text{ cm}^{-1}$  and  $1650$   
22  $\text{cm}^{-1}$ ) were generated (Supplementary Fig. 8). The corresponding single wavenumber images  
23 present the spatial arrangements of these intensity distributions (Supplementary Fig. 8). It

1 can be seen that, like the Bionate II 80A specimen subjected to the B20 method, there is  
2 considerable variation in the levels of material degradation detected within the sample.

### 3 **3.3 Univariate analysis of Bionate II (*in vivo* degradation)**

4 ATR mapping was also applied to a PCU component (Supplementary Fig. 9) (Bionate II 80A,  
5 DSM, Heerlen, Netherlands) explanted from a BDyn device. Mapping of *in vivo* specimens  
6 provides a unique insight into the authenticity of *in vitro* oxidation methods, particularly  
7 with respect to the spatial heterogeneity of degradation. The selected area of the PCU ring  
8 component (Supplementary Fig. 10) was magnified with  $1174\text{ cm}^{-1}$ ,  $1248\text{ cm}^{-1}$  and  $1650\text{ cm}^{-1}$   
9 single wavenumber colour maps overlaid (Fig. 5). Each map was acquired by defining an  
10 acquisition matrix with regularly spaced acquisition points, where the acquisition step size  
11 was larger than the detector aperture size. While such a method does not result in a  
12 spatially contiguous image, it does allow for the spectroscopic assessment of larger  
13 specimen regions, with a relative reduction in acquisition time, allowing for use in time-  
14 sensitive studies. The hyperspectral chemical maps revealed that degradation of the *in vivo*  
15 specimen is spatially diverse, as was also found with *in vitro* degradation methods. Further,  
16 the actual spatial arrangement of the degradation appears to follow a trend with respect to  
17 the PCU ring radial position. This was found to be the case for the formation of the aromatic  
18 amine peak at  $1650\text{ cm}^{-1}$ , the C-C cross-linking peak at  $1174\text{ cm}^{-1}$  and the disappearance of  
19 the C-O-C carbonate peak found at  $1248\text{ cm}^{-1}$  (Fig. 5), all of which are reported identifiers of  
20 PCU oxidative degradation. It should be noted that the regions exhibiting  $1650\text{ cm}^{-1}$   
21 aromatic amine formation do not perfectly coincide with the presence of  $1174\text{ cm}^{-1}$  C-C  
22 cross-linking (Fig. 5).



1

2

Figure 5. *In vivo* explant with single wavenumber  $1174\text{ cm}^{-1}$ ,  $1248\text{ cm}^{-1}$  and  $1650\text{ cm}^{-1}$  maps overlaid. All

3

images scaled equally (height and width).

4

### 3.4 Multivariate analysis (Difference Spectrum Projection)

5

Multivariate analysis was conducted according to the methodology section. The mean

6

spectra of Bionate II 80A specimens (B0 untreated and B20 treated) are presented (Fig. 6).

7

These spectra were defined as reference undegraded and degraded spectra respectively and

8

were used to calculate a degradation difference spectrum (subtraction of the former from

9

the latter) (Fig. 6). It can be seen that peaks reported in the literature as being indicative of

10

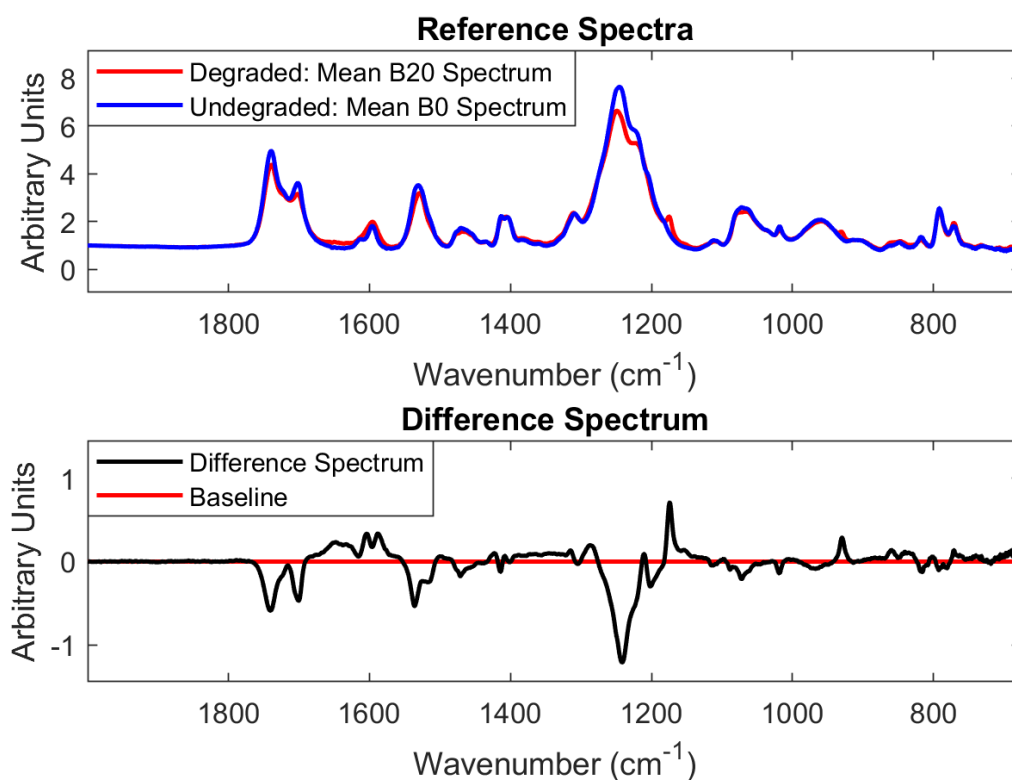
PCU degradation (the emergence of peaks at  $1174\text{ cm}^{-1}$  and  $1650\text{ cm}^{-1}$ , in addition to the

11

decline of a peak at  $1248\text{ cm}^{-1}$ ) are highlighted in this degradation difference spectrum by

12

being above or below the baseline as appropriate.



1

2

**Figure 6. Undegraded and degraded reference spectra (top) and difference spectrum calculated from the subtraction of undegraded reference from degraded reference, including baseline (bottom).**

3

4 The *in vitro* hyperspectral chemical images were projected along the degradation difference

5 spectrum, which applied weights to each wavenumber according to the difference spectrum

6 in Figure 6 and summed the resultant values. The output values were used to generate

7 degradation index maps (Fig. 7). A histogram was also generated and used to investigate the

8 distribution of the degradation indices across all images. Regions of the histogram (Fig. 7)

9 were thresholded where populations were separable. The mean spectra of all pixels

10 assigned to each class are presented (Fig. 8, bottom right). Through these mean spectra, it

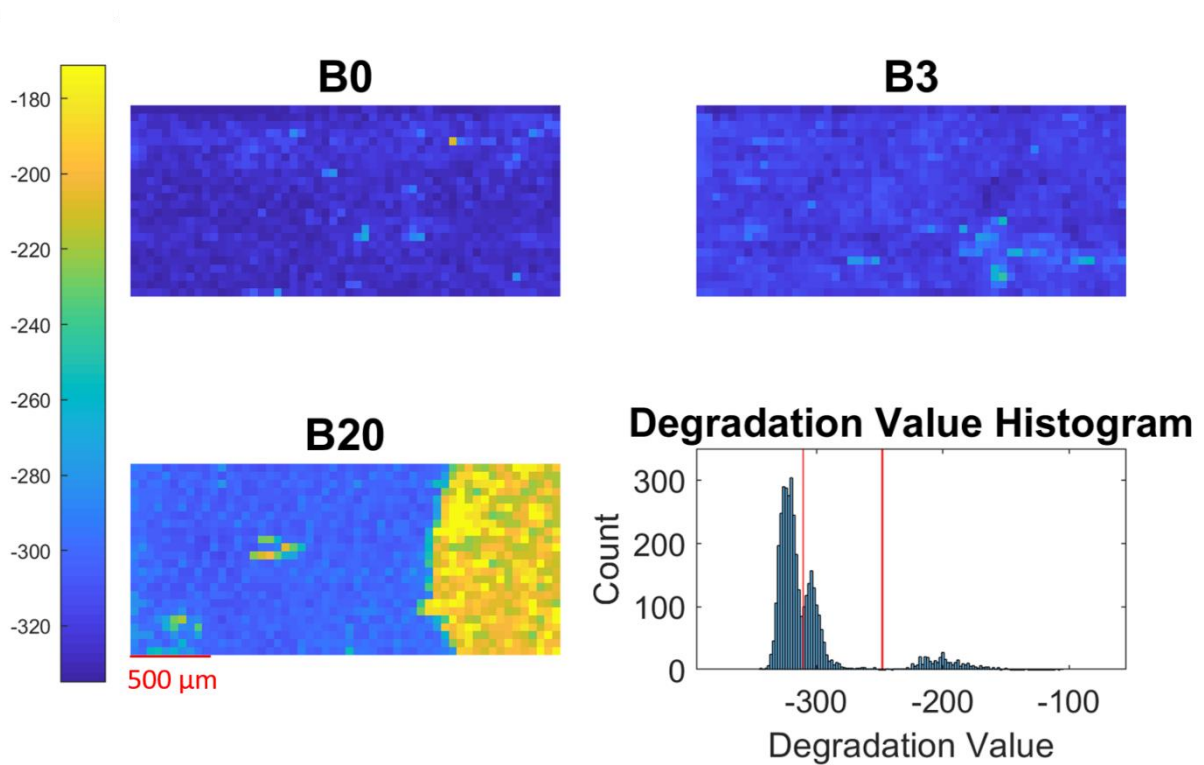
11 was found that the classes represented (1) undegraded, (2) degraded and (3) very degraded

12 regions, identified according to the magnitude of the degradation peaks of interest reported

13 in the literature. Class 1 (undegraded, blue colour in Fig. 8) exhibited a large peak at 1248

14  $\text{cm}^{-1}$  and no peaks at  $1650 \text{ cm}^{-1}$  and  $1174 \text{ cm}^{-1}$ . Class 2 (degraded, red colour in Fig. 8)

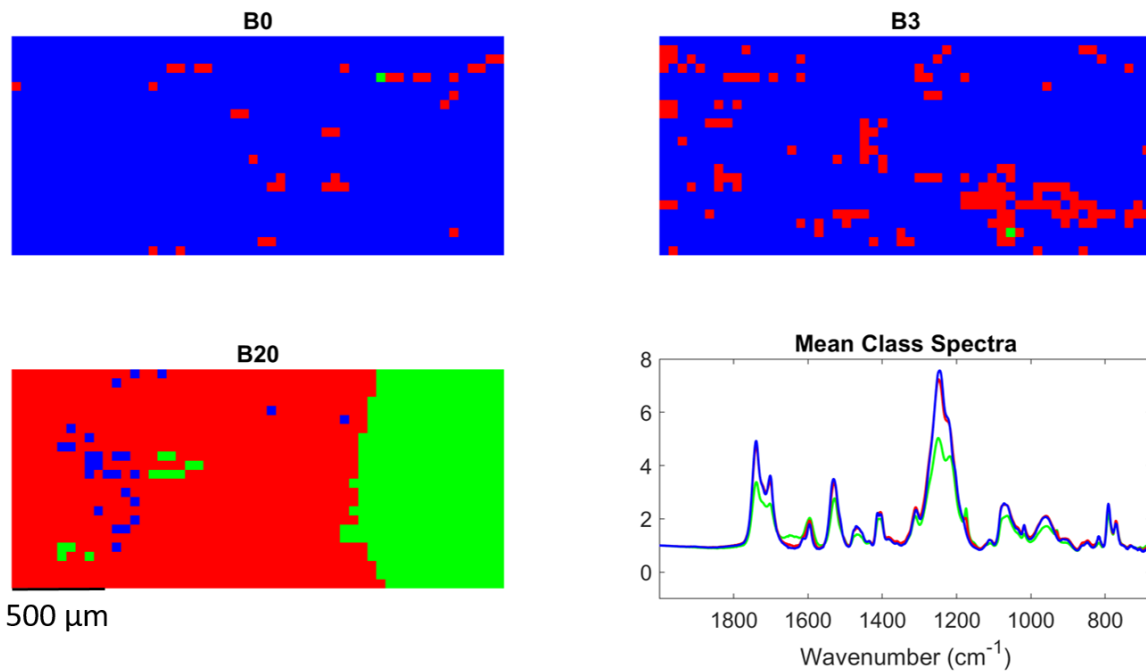
1 exhibited a slightly smaller peak at  $1248\text{ cm}^{-1}$ , increased absorption at  $1650\text{ cm}^{-1}$  and a  
2 small but distinctive peak at  $1174\text{ cm}^{-1}$ . Class 3 (very degraded, green colour in Fig. 8)  
3 exhibited a smaller peak at  $1248\text{ cm}^{-1}$  and larger peaks at  $1650\text{ cm}^{-1}$  and  $1174\text{ cm}^{-1}$ . Several  
4 pixels representing degradation were found in the control specimen and the specimen  
5 exposed to B3 oxidation (see red and green pixels in Figure 8), although more were present  
6 in B3 than B0. Conversely, some undegraded pixels were found in the specimen exposed to  
7 B20 oxidation.



8



1 **Figure 7. Degradation value images produced by projection of HCIs along degradation difference spectrum,**  
2 **in addition to accompanying histogram. All images scaled equally (height and width). Image colour scale**  
3 **from 1<sup>st</sup> to 99<sup>th</sup> percentile. Threshold bars added to histogram.**



4  
5 **Figure 8. B0, B3 and B20 degradation classification images (top left, top right, bottom left) with colour-coded**  
6 **classification mean spectra (bottom right). All images scaled equally (height and width).**

#### 7 **4. Discussion**

8 Through a novel application of HCI, this study demonstrates spatial changes in degradation  
9 across PCU biomaterial surfaces. It was found that it is possible to identify regional  
10 differences in chemical degradation, allowing for more detailed investigations. The results  
11 call into question the validity of the use of traditional point spectroscopic methods by  
12 illustrating that, in materials where heterogeneity in surface degradation exists, the  
13 acquisition of just one spectrum runs the risk of being unrepresentative of a specimen as a  
14 whole. A single spectrum can lead to subjective results as the location of spectral  
15 acquisitions can have a large effect on the ultimate conclusions drawn. The present study

1 has also shown that even in instances where large hyperspectral chemical images are  
2 acquired, a single mean spectrum might not represent a material as a whole. Further, a  
3 mean spectrum calculated for a large area cannot identify any sub-region of the material, as  
4 a mean spectrum is a linear combination of all, possibly very distinct, collected spectra  
5 which can result in a “blending” effect. Therefore accurate conclusions can only be drawn  
6 from HCl when combined with appropriate data processing techniques. For example, in a  
7 previous study which used point acquisition to examine chemical changes, PCUs exposed to  
8 3% H<sub>2</sub>O<sub>2</sub> *in vitro* oxidative treatment showed no evidence of hard segment degradation in  
9 any of the specimens evaluated [24]. Had point spectroscopy been utilised in the present  
10 study, similar conclusions would have been drawn. However it has been demonstrated,  
11 through the generation of select wavenumber images [1174cm<sup>-1</sup>, 1248cm<sup>-1</sup> and 1650cm<sup>-1</sup>]  
12 and associated histograms, that in some localised regions of the PCU specimen exposed to  
13 an identical treatment (B3), a peak emerges at 1650 cm<sup>-1</sup> (indicating aromatic amine),  
14 implying the local occurrence of hard segment degradation [12,24]. Further spatial  
15 heterogeneity of degradation was observed in the case of the untreated control specimens  
16 (B0) as well as the more harshly oxidised specimens (B20). Without HCl, these chemically  
17 distinct regions would go unnoticed, or would be over-represented. The observation of  
18 locally distinct chemical regions on the B0 control specimen was unexpected and may have  
19 been caused by one or more factors including, potential inconsistencies in the biomaterial  
20 mixing/formulation/manufacture process and/or in the sterilisation of the PCU plaques. It  
21 may also be caused by mechanical factors. At this time the hard segment changes cannot be  
22 attributed to a single factor. Both Trommsdorff et al. [40] and Cipriani et al. [9] stated that,  
23 as the degradation is limited to the surface layer, the functionality [40] and the mechanical  
24 properties [9] of the entire device are unlikely to be affected. However, Lawless et al. [8]

1 discovered that the viscous property, of the BDyn device's PCU component, was statistically  
2 different, at specific frequencies, after *in vitro* degradation [8].

3 Ultimately, a balance between efficiency and accuracy should be struck and with that  
4 sentiment in mind, hyperspectral chemical mapping was presented with *in vivo* degraded  
5 explanted specimens. While such a method does not result in a spatially contiguous image,  
6 it does allow for the spectroscopic assessment of larger specimen regions, with a relative  
7 reduction in acquisition time, allowing for use in time-sensitive studies. The hyperspectral  
8 chemical maps of the *in vivo* degraded PCU component revealed that degradation is  
9 spatially diverse; this was also found with *in vitro* degraded PCU specimens. Further, it was  
10 discovered that the regions exhibiting  $1650\text{ cm}^{-1}$  aromatic amine formation (indicative of  
11 hard segment degradation [12,24]) do not perfectly coincide with the presence of  $1174\text{ cm}^{-1}$   
12 C-C cross-linking (indicative of soft segment degradation [12,24]) as was found to be the  
13 case with *in vitro* degraded specimen, suggesting a more complex degradation mechanism  
14 than simple oxidation. In addition, it was observed that degradation heterogeneity  
15 appeared to be somewhat radially dependent, possibly due the introduction of localised  
16 mechanical stresses or enzyme action. However the cause is not currently known.

17 Finally, a simple multivariate method was developed coined "difference spectrum  
18 projection". This method generated and assigned a degradation index to each pixel of the *in*  
19 *vitro*-degraded PCU biomaterial specimen HCLs. Upon inspection of the degradation index  
20 distribution, it was found that the specimens comprise regions drawn from one of three  
21 distinct "degradation-level" populations. Despite the use of three different degradation  
22 protocols (including one control), heterogeneity in degradation-levels was found across the  
23 surface of each specimen, again emphasising the importance of HCL techniques. The

1 multivariate technique used provides many advantages over standard univariate analysis,  
2 not least: significant reductions in operator bias. It should be noted that after reference  
3 spectra are selected according to the method, all steps described required can be  
4 automated. The difference spectrum generated also has the potential to expose spectral  
5 peaks or broad bands which might not be as readily visible to a human operator and is  
6 further capable of using multiple identifier peaks in unison.

## 7 **5. Conclusion**

8 This study demonstrates the potential of hyperspectral chemical imaging (HCI) in the  
9 detection of spatial variation of the degradation of PCU biomaterials. The use of HCI can  
10 prevent the unavoidable introduction of subjectivity associated with traditional point  
11 spectroscopic methods and its use will lead to a deeper understanding of a biomaterial's  
12 chemical condition in pre-implantation quality control, *in vitro* degradation studies and  
13 retrieval studies. It is proposed that it is now time to move the standard forward, from that  
14 of point acquisition to that of HCI.

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18 and 335508).

## 19 **Author contributions**

20 R.M.D. and B.M.L. performed hyperspectral chemical imaging of all specimens. B.M.L. and  
21 H.E.B. performed all *in vitro* degradation of the polycarbonate urethanes and B.M.L., R.M.D.,

1 D.M.E. and D.E.T.S. contributed to the design of the degradation experiments. R.M.D. and  
2 A.A.G. produced all figures and performed all data analysis within this study. R.M.D., B.M.L.  
3 and A.A.G. contributed to the writing of this article. All authors reviewed and edited the  
4 manuscript.

5 **Hyperspectral chemical image data, Matlab functions and scripts** are available from the  
6 authors.

7 **Correspondence and requests** should be made to A.A.G.

8 **How to cite this article:** Dorrepaal, Lawless *et al.* Hyperspectral chemical imaging reveals  
9 spatially varied degradation of biomaterials.

#### 10 **Conflict of Interest**

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13 604935). No employees of S14 Implants were involved in this study and no benefit of any  
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