# Journal of Materials Chemistry B

Accepted Manuscript




This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/materialsB

# Physicochemical Characterization of Segmented Polyurethanes Prepared with Glutamine or Ascorbic Acid as Chain Extenders and their Hydroxyapatite Composites

S. M. Cetina-Diaz[1], L. H. Chan-Chan[1], R. F. Vargas-Coronado[1], J.M. Cervantes-Uc[1], P. Quintana-Owen [2] K. Paakinaho [3], M. Kellomaki [3], L. Di Silvio[4], S. Deb[4], J.V. Cauich-Rodríguez[1]

[1]Centro de Investigación Científica de Yucatán A.C
Calle 43 130, Col. Chuburná de Hidalgo, C.P. 97200, Mérida, Yucatán, México
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional
Unidad Mérida Km 6 Antigua Carretera a Progreso, C.P. 97 310, Mérida, Yucatán, México
[3]Department of Electrical and Communcations Engineering and BioMediTech., Tampere University of Technology, Tampere, Finland
[4]King's College London Dental Institute, Floor 17 Guy's Tower, Guy's Campus
London SEI 9 RT, UK

Journal of Materials Chemistry B Accepted Manuscript

## ABSTRACT

The development of elastomeric, bioresorbable, and biocompatible segmented polyurethanes (SPUs) for use in tissue-engineering applications has attracted considerable interest in recent years because of the existing need of mechanically tunable scaffolds for regeneration of different tissues. In this study segmented polyurethanes were synthesized from poly (ε-caprolactone)diol, 4,4'-methylene bis(cyclohexyl isocyanate) (HMDI) using osteogenic compounds such as ascorbic acid (AA) and L-glutamine (GL) as chain extenders, which are known to play a role in osteoblast proliferation and collagen synthesis. Fourier Transform Infrared spectroscopy (FTIR) revealed the formation of urethanes linkages at 3373, 1729, 1522 $cm^{-1}$ (N-H stretching, C=O stretching, N-H bending and C-N stretching vibrations respectively) while urea formation was confirmed by the appearance of a peak at 1632 $cm^{-1}$. Differential scanning calorimetry, dynamic mechanical analysis, X-ray diffraction and mechanical testing of the polyurethanes showed that these polyurethanes were semi crystalline polymers (Tg=-25°C; Tm=51.4-53.8°C; 2θ=21.3° and 23.4°)

exhibiting elastomeric behavior (ε>1000%) only for those prepared by HA incorporation during prepolymer formation.

Dense and porous composite matrices of the segmented polyurethanes were prepared by the addition of hydroxyapatite (HA) via either mechanical mixing or *in situ* polymerization and supercritical fluid process, respectively. The addition of HA by physical mixing decreased crystallinity (from 38% to 31%) of the composites prepared with ascorbic acid as chain extender. Both $T_g$ of composites and the strain were also lowered up from -38-36°C and 27-39% for ascorbic acid and glutamine containing polyurethanes respectively. Composites prepared with ascorbic acid as chain extender yielded higher Young´s modulus and tensile strength than composites prepared with glutamine when HA was incorporated during prepolymer formation. Composites obtained by incorporation of HA by physical mixing revealed a poor dispersion in comparison to composites obtained via HA inclusion during prepolymer formation. In contrast, good dispersion of HA and porosity were achieved at 60°C, 400 bar and holding times between 0.5 h and 2 h with a down time between 15 min and 60 min in the $CO_2$ reactor. Biocompatibility studies showed that SPU´s containing ascorbic acid allowed alveolar osteoblast proliferation increasing, hence, potentially suitable for bone tissue regeneration

**Keywords**: Polyurethane, hydroxyapatite, composite, scaffold.

Journal of Materials Chemistry B Accepted Manuscript

## 1 INTRODUCTION

The design and development of clinically relevant scaffolds for bone tissue engineering continues to pose challenges despite an increasing research interest in this field. The scaffold in bone tissue engineering plays a key role, which provides the temporary architecture for the reparative process. Thus biodegradable polymers both from natural and synthetic origin that are cytocompatible are of interest in the design scaffolds for bone tissue engineering [1]. With extensive research in this field [2,3] it has become clear that biocompatibility of the scaffold material is imperative, a matrix with controlled degradation is desirable and the mechanical strength of the material should match those of either cortical or trabecular bone at the beginning or not collapse or alter under physiological loads and through the duration till bone tissue formation occurs. In addition, the sterilization should not alter the properties and consideration should be given to the porosity of the scaffold. Bone loss can occur for numerous reasons, hence, the need exists in the field of orthopaedics, dentistry and maxillofacial surgery, which generates bone defects of varying sizes and locations in the body leading to the need of being able to develop scaffolds that can be tailored to suit [4,5].

These requirements can be fulfilled by employing biodegradable polyurethanes as they exhibit controlled degradation, a wide range of mechanical properties and good biocompatibility achieved through the use of biodegradable soft segments or labile linkages on the rigid segment [4,6]. The properties of these matrices can be further improved by incorporating various types of bioactive fillers, like hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) [7-9] and various bioactive glasses [10]. Biodegradable elastomeric segmented polyurethanes such as those prepared with 1,4-diisocyanatobutane, poly (ε-

Journal of Materials Chemistry B Accepted Manuscript

caprolactone) (PCL) as the biodegradable macrodiol and tyramine-1,4- diisocyanatebutane as biodegradable chain extender, were reported to exhibit substantial improvement in storage modulus that ranged from 52 to 278 MPa by increasing the molecular weight of the PCL [11]. Similarly, composite materials based on polyurethane (pentaerythritol prepolymers terminated with ethyl 2,6-diisocyanate hexanoate or glycolic acid) containing 10% calcium phosphate (β-TCP) were found to increase compressive strengths from 2 to 2.6 GPa that also had increased osteoblast viability [12].

Furthermore, injectable formulations proposed by Adhilkari et al [13] were based on a combination of pentaerythritol prepolymers terminated with ethyl 2,6-diisocyanate hexanoate with either glycolic or lactic acid. SPU foams based on HMDI-PCL-Castor oil-BDO(1,4 butanediol) with up to 50 wt.% of HA and exhibiting 82% porosity (average pore size of 510 μm) were also proposed [14]. Nano-HA composites in polyurethane matrix synthesized from HMDI, poly(ethylene glycol), castor oil and 1,4-butandiol for guided bone regeneration have been reported by Liu et al [15] with tunable properties to adapt for engineering of tissues. Composites made from porous polyurethanes (HDMI / PCL / [EG)(Ethylene glycol)] and Bioglass® (5 to 20%) were reported by Ryszkowska et al [10] with an increase in the storage modulus (0.17 to 0.81 MPa) and improvements in bioactivity after the formation of an apatitic layer. In a similar study [16] on polyurethanes/poly(vinyl) alcohol/Glass nanoparticles composites were reported however with mechanical properties that were still low for applications that require the reproduction of morphology and mechanical properties of mature trabecular bone, but these properties were good enough to support cell growth and proliferation.

Improvements in mechanical properties of polyurethanes for bone tissue regeneration have also been proposed by Bil et al. by increasing their hard segment content from 20 to 70%

Journal of Materials Chemistry B Accepted Manuscript

[17]. In this case, by increasing the content of hard segments, phase separation occurred which led to the decrease in the osteogenic potential of human bone-derived cells.

Despite several modifications of polyurethanes to develop them as scaffolds for engineering tissues, osteogenic molecules that play a role in osteoblasts proliferation [18] and collagen synthesis and are commonly found in cell culture media have not been used in the synthesis of segmented polyurethanes except for the work of Zhang et al. [19] who used ascorbic acid as part of the prepolymer but not as chain extender. In this study we report the synthesis, characterization and properties of biodegradable segmented polyurethanes based on PCL, HMDI and either ascorbic acid (AA) or glutamine (GL) as chain extenders. Furthermore to enhance the bone forming potential of these scaffolds, hydroxyapatite (HA), a known osteoconductive material, was incorporated during prepolymer formation (*in situ,* I) and by physical mixing of a SPU solution with a HA suspension (mixed, M) to yield composites. With porosity in scaffolds being essential for nutrient transport, porous forms of the matrices developed were also developed using a supercritical $CO_2$ reactor.

## 2 Materials and methods

### 2.1. Polyurethane synthesis

Poly (ε-caprolactone) diol (PCL diol, $M_n$=2000), 4,4(metylene-bis-cyclohexyl)isocyanate (HMDI), ascorbic, acid, L glutamine, and stannous octoate were purchased from Sigma-Aldrich (Milwaukee, USA). Dimethyl formamide (DMF) from Sigma-Aldrich (Steinheim, Germany), Tetrahydrofurane (THF) from JT Baker (Phillipsburg, USA) were used as solvents in the synthesis or film preparation. Composites were prepared with hydroxyapatite from Merck (Darmstadt, Germany) with particle size distribution of 4.2 ± 2.3 μm as measured by a Coulter LS 100Q analyzer.

Journal of Materials Chemistry B Accepted Manuscript

Segmented polyurethanes (SPUs) with a molar radio of 1:2.05:1.05 (PCL:HMDI:GL/AA) were prepared by a two-step procedure in nitrogen atmosphere at 60°C with 0.3% w/w stannous octoate as catalyzer. In the first step, PCL diol terminated (4mmol, $M_n$=2,000) and catalyzer were dissolved in dimethylformamide (DMF). Next, the solution was mixed with an excess of HMDI (8.2 mmol) and stirred during 4 hours in order to form an NCO-terminated prepolymer. In the second stage, either L-glutamine (4.2 mmol) or ascorbic acid (4.2 mmol) was dissolved in DMF and added to the reaction and stirred during 2 hours to extend the polymer. To stop the reaction, it was precipitated and washed with distilled water. Finally the polymer was dried at 60ºC at reduced pressure. The SPUs obtained will be referred as SPUGL with glutamine and SPUAA with ascorbic acid as the chain extenders. Scheme 1a depicts the possible reaction mechanism in order to form linear segmented poly (urethane-ureas). Scheme 1b shows the possible crosslinking reaction considering the multifunctional nature of the chain extenders.

Model polyurethanes (not segmented) were synthesized in one step by mixing catalyzer and solutions of HMDI (4 mmol) with solution of glutamine or ascorbic acid (4 mmol). Reaction was carried at the same procedure and conditions than SPUs to show the feasibility of segmented polyurethane synthesis.

### 2.1.1 Composite preparation

HA composites ranging from 5 to 20 wt.% of the ceramic were prepared in preliminary studies. From these studies, 20 wt.% (approximately 6 percent by volume) was selected not only as it has been reported that high amounts of HA promotes lower degradation rates[14] but also because of this composition was expected to provide a good balance between processability, mechanical properties and biocompatibility. Then, composites with 20 wt.%

Journal of Materials Chemistry B Accepted Manuscript

HA were prepared by either mixing a THF suspension of HA with the preformed SPU solution (referred as SPU-M) or by adding HA during prepolymer formation (referred as SPU-I). Dense films were obtained after casting the composites in Teflon moulds.

Porous composites were obtained by placing the freshly made SPU-I in a supercritical $CO_2$ reactor varying the holding time (0.5 h and 2 h) and the downtime (0.25 h and 1 h) keeping the temperature at 60°C and the pressure at 400 bar.

## 2.2. Characterization of SPUs and composites

### 2.2.1. Physicochemical characterization

$^1$H NMR and Fourier transform infrared (FTIR) spectroscopy

Proton nuclear magnetic resonance spectra were obtained with a 300 MHz Varian spectrometer (Palo Alto, CA) using deuterated chloroform as solvent (3 mg/ml) and tetramethylsilane as internal standard.

Infrared spectra of the SPU´s were obtained after casting a film on KBr disc with a Nicolet Protégé 460 FTIR (Madison, WI) in the spectral range from 4000 to 400 $cm^{-1}$ averaging 50 scans with a resolution of 4 $cm^{-1}$.

Molecular weight was determined by gel permeation chromatography (GPC) using an Agilent 1100 GPC-SEC system equipped with Zorbax PSM (60S and 1000S) coupled columns and a refractive index detector (Agilent technologies, Germany). DMF HPLC grade, without LiCl, was used as eluent with a flow rate of 1ml/min at 50°C and the calibration curve was obtained with 1 mg/ml polystyrene molecular weight standards in the range from 1,050 to 420,600 g/mol dissolved in DMF.

Journal of Materials Chemistry B Accepted Manuscript

### 2.2.2. Thermal properties

The thermal behavior was evaluated with a DSC 7 from Perkin Elmer (Norwalk, CT) using 5 mg of the polymer encapsulated on aluminum pans. The polymer was heated from 40ºC to 160ºC at 5ºC/min under nitrogen atmosphere. First and second thermograms were recorded. Relative percent crystallinity (Xc) of the PCL in the SPUs was determined from the enthalpy of fusion using the following equation:

$$\%Xc = \frac{\Delta H_f}{w_{ss} \times \Delta H^\circ_f} x100 \quad (1)$$

where $\Delta H_f$ is the enthalpy of melting of SPUs obtained experimentally, $w_{ss}$ is the theorical mass fraction of the flexible segment and $\Delta H^\circ_f$ is the enthalpy of 100% crystalline PCL taken as 136 J /g [20].

For thermogravimetric analysis (TGA), 20 mg of the samples were heated from 50ºC to 700ºC at 10ºC/min under nitrogen atmosphere using a TGA 7 from Perkin Elmer (Norwalk, CT). From the first derivative, decomposition temperatures ($T_d$) were obtained. The glass transition temperature ($T_g$) was obtained by dynamic mechanical analysis with a Perkin Elmer DMA 7 (Norwalk, CT) in the extension mode. Strips of 20 x 3 x0.1 mm were heated from -100ºC to 100ºC at 5ºC/min using a static force of 90 mN and a dynamic force of 70 mN at 1 Hz.

### 2.2.3. Microstructure determination

**X- ray diffraction (XRD)**

X ray diffraction measurements were carried out with a D-5000 Siemen diffractometer (Karlsruhe, Germany) using monochromatic radiation ($CuK_\alpha$ $\lambda$=1.5418 Å) at 35 kV and 24

Journal of Materials Chemistry B Accepted Manuscript

mA. For these experiments, 1 $cm^2$ films were used and registered in the range 5º<2θ<60º with a step count of 3 s and a step size of 0.02º (2θ).

**Scanning electron microscopy (SEM)**

Microstructure of the dense and porous SPUs composites was observed by SEM using a Jeol 6360 LV (Tokyo, Japan). Samples were gold coated and observed using an accelerating voltage of 20 kV.

### 2.2.4. Mechanical properties

Tensile mechanical properties were obtained using rectangular specimens of 10 x 5 x 0.1 mm with a Minimat testing machine (Kyoto, Japan) using a cross-head speed of 50 mm/min according to ASTM D-412. The Young´s modulus or/and Young´s modulus at 100% ($E_{100}$), tensile strength (σ) and strain to failure (ε) are reported.

### 2.5. *In vitro* degradation

Accelerated degradation was conducted using 2 N HCl, 5 M NaOH and $H_2O_2$ (30% v/v). For these experiments, a known amount of SPUAA, SPUGL, SPUAA-I and SPUGL-I was heated for 24 h under refluxing conditions. *In vitro* degradation studies were also conducted in phosphate buffer saline (PBS, pH=7.4) at 37°C for six months. Polymer films (2 x 3 cm), with an approximately mass of 50 mg were used. PBS was changed every week to keep the same concentration.

Mass loss was calculated by the equation:

$$\%mass loss = \frac{m_f - m_i}{m_i} x100 \qquad (2)$$

Journal of Materials Chemistry B Accepted Manuscript

Where $m_i$ is the weight of the polymer after degradation or incubation and $m_f$ is the weight of the material after drying at 60°C in a vacuum oven during 24 h [21].

### 2.5.1 *In vitro* cytocompatibility studies

#### 2.5.1.1 Osteoblast cell culture

Alveolar human osteoblast cells (HOB) were used for biocompatibility studies. Cells were cultured in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% foetal calf serum (FCS), 1% non-essential amino acids, L-ascorbic (0.150 g/l), 1% of 200 mM L-glutamine (2 mM), 2% of 1 M HEPES, penicillin (100 U/ml) and streptomycin (0.1 mg/ml) (all from Sigma, UK). HOBs were cultured at 37°C in a controlled humid atmosphere with 5% $CO_2$.

#### 2.5.1.2 Indirect *MTT* toxicity assay

The MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide] assay was used as an 'indirect' method to assess any potential cytotoxic leachables from SPUAA, SPUGL, SPUAA-I, SPUGL-I only, as those composites prepared by mixing were not different to the pristine matrix and pure HA. Sterile SPUs scaffolds were placed in 3 ml of HOB medium (DMEM) for elution studies. Eluants were collected from SPUAA, SPUGL for 24 and 48 h and cells were exposed for 24 and 72 h. The negative, non-toxic control, was DMEM and the positive toxic control was 10% ethanol. Following incubation, MTT was added to all the wells, incubated for a further 4 h at 37°C; the insoluble formazan salt produced was dissolved using 100 µl dimethyl sulfoxide (DMSO, Sigma D2650- tissue culture grade). The plates were gently agitated for 5 min to ensure complete crystal dissolution and optical

Journal of Materials Chemistry B Accepted Manuscript

densities were measured at a test wavelength 570 nm, subtracting background absorbance at reference wavelength 620 nm (Dynex Technologies, Chantilly, VA)

#### 2.5.2.6 Cell proliferation study (Alamar Blue™)

Proliferation of cells was determined using the Alamar Blue™ assay (Life technologies) on three replicate samples of SPUAA, SPUAA-I, SPUGL, SPUGL-I. Tissue culture plastic (TCP) and medium supplemented with 10% ethanol were used as negative (non-toxic) and positive (toxic) controls, respectively. Proliferation was measured at 1, 3, 7 and 14 days post seeding. The percentage of cell viability was calculated considering that TCP was 100%.

### Data analysis

Data were presented as the mean ± standard deviation. Statistical significance was calculated using one way analysis of variance (ANOVA) followed by Student's t-test ($p < 0.05$ was considered significant).

## 3 RESULTS

### Spectroscopic studies

The chemical structure of the polyurethane SPUAA or SPUGL was elucidated both by $^{1}H$ NMR and FTIR. $^{1}H$ NMR spectra of SPUGL and SPUAA (Figure 1) showed several similarities as PCL is the major component. This analysis showed characteristic peaks of the polyol, which consist of PCL (4.05, 2.31, 1.67, 1.37 ppm) and a smaller amount of ethylene glycol (4.22 and 3.69 ppm). The signals of diisocyanate protons were observed

Journal of Materials Chemistry B Accepted Manuscript

between 0.5 and 2 ppm. Additionally, protons adjacent to urethane group at 3.39 ppm confirm that the reaction occurred.

The IR spectra of HA, SPUs and HA/SPUs composites prepared with ascorbic acid (a) and glutamine (b) are shown in Figure 2. The absorption peak at 3373 $cm^{-1}$was assigned to N-H vibrations while $CH_2$ asymmetric and symmetric stretching vibrations were observed at 2946 and 2869 cm-$^{1}$, respectively. Carbonyl stretching (C=O) appeared between 1729 and 1760 $cm^{-1}$ which include the ester group from the PCL and the urethane group (NHCOO). Amide II absorption (urethane N-H bending + C-N stretching) was located at 1522 $cm^{-1}$ while the peak at 1166 $cm^{-1}$ was attributed to C-O-C stretching vibration in the soft segment. The peak at 1635 $cm^{-1}$ was assigned to the urea linkages confirming the reaction of amine groups from the glutamine with NCO although the presence of water might also contribute. FTIR spectra (figure not shown) of model polyurethanes obtained from HMDI and the chain extender (1:1) allowed to confirm that glutamine reacted with the diisocyanate to form urea linkages while the carboxylic acid groups reacted to form an unstable anhydride group resulting in amide formation. The polyurethanes obtained from HMDI and ascorbic acid showed urethane link formation.

The presence of HA in these composites was verified by peaks at 565, 603 and 1032 $cm^{-1}$ ($PO_4^{3-}$), at 1468 $cm^{-1}$ ($CO_3^{2-}$) and 3565 $cm^{-1}$ corresponding to the vibration of hydroxyl ions ($OH^-$).

**Thermal properties**

All types of SPUs and HA/SPUs composites containing PCL as soft segmented were semicrystallyne materials as they exhibited melting and endotermic peaks around 50°C and 53°C (see supplementary data S1). This is attributed to crystalline phase of the PCL as

Journal of Materials Chemistry B Accepted Manuscript

these aliphatic polyurethanes do not tend to crystallize. The presence of a melting peak suggested that the crystalline structure of the PCL is preserved due to its relatively high molecular weight (c.a. Mn=2000). Surprisingly, the inclusion of HA in the composite tends to reduce its crystallinity (obtained from DSC) from 40% to 31% in SPUAA-M but had no effect of other formulations.

The tan δ variation with temperature showed a well-defined α transition which was related to the soft segments of the SPU. For SPUAA and SPUGL this was located at -25°C and was reduced, up to -33°C when HA was incorporated during prepolymer formation (SPUAA-I) but up to -36°C when HA was added by mechanical mixing (SPUAA-M). This unexpected behaviour is in contrast to those reported by Liu *et al.* [14] who observed an increase in Tg with HA addition. The shift in the $T_g$ of the phase amorphous PCL suggests that the filler and the rigid segment content is restricting PCL chain movement as the $T_g$ of pure PCL was found between -65ºC and -60°C. For SPUGL the reduction in $T_g$ was only observed when the HA was incorporated after polymer formation.

Despite crystallinity of the SPUAA-I was slightly higher than that of SPUAA, Tg decreased only 8.8°C by the addition of HA. The slight increase in crystallinity will only reduce the intensity of the Tan δ peak taken as the Tg of the polyurethane but not the actual peak position because there is no interaction between the filler and the matrix.

Thermogravimetric analysis of the neat polymers showed a main decomposition temperature at 396-397°C which was attributed to the decomposition of the flexible segment (PCL). This temperature increased by adding HA with the effect being more pronounced when the filler was added during the prepolymer synthesis. Small decomposition peaks were also detected at higher temperatures (492-493°C) which were

Journal of Materials Chemistry B Accepted Manuscript

assigned to the rigid segments [22] and based on the thermal behaviour of model polyurethanes synthesized. The improvement of thermal stability can be explained due to the interactions between HA and SPUs matrix i.e. hydrogen bond formation between the urethane (-NHCOO-) of SPUs and –OH of hydroxyapatite. The lower rate of mass loss of HA/SPU-I compared to HA/SPU-M (18%/t vs. 20%/t) can be explained if one considers the possible reaction between hydroxyapatite and the HMDI during prepolymer formation. Table 1 summarizes the thermal properties of SPU and their SPU/HA composites.

**Microstructure by XRD and SEM**

Figure 3 shows the XRD patterns of HA, SPUs and HA/SPUs composites. The semi-crystalline nature of both SPUAA (Figure 3a) and SPUGL (Figure 3b) can be confirmed from the presence of reflections at 2θ =21.3°, 22.0° and 23.4° from the PCL soft segments. This behavior is common in polyurethane synthesized with PCL diol 2000 g/mol which tend to be semicrystalline, compared to polyurethanes synthesized with low molecular weight PCL, which tend to be more amorphous [23-24]. By XRD it was also observed that the diffraction pattern of the HA (2θ = 25.8°, 31.8°, 32.80°, 34.12° and 39.6°) was retained after its incorporation to the SPU either by mechanical mixing or during prepolymer formation. Based on this, it can be said that there is good dispersion during incorporation of the HA and these results indicate that the osteoconductive potential of HA is maintained during their incorporation into the SPU's.

Figure 4 show the SEM images of the SPU's and composites SPU/HA. The formation of agglomerates (spherullites) in the neat polyurethanes is evident and its sizes were smaller in glutamine containing polymers. When the HA was incorporated into the polyurethanes during prepolymer formation these structures were no longer visible. However, a good

Journal of Materials Chemistry B Accepted Manuscript

dispersion was observed in those SPU's prepared with ascorbic acid while rich HA areas were observed in glutamine containing polyurethanes (see Figures 4c and 4d, respectively). In SPU/HA composites prepared by mechanical mixing (Figures 4e and 4f) it can observe that the agglomerates grain size increase but retaining a ceramic homogeneous dispersion.

In contrast to the dense structure observed by polymer casting, a porous structure was obtained by supercritical $CO_2$. SEM images of the porous composites are shown in Figure 5 where a fairly even distribution of pores in the matrix can be seen. By means of this technique a macroporous scaffold with smaller pores mostly in the walls was obtained which make them suitable for bone tissue engineering. Hydroxyapatite particles are not easily observed but a closer inspection showed that it still agglomerates. Porosity is thought to be modified by the variables in the supercritical $CO_2$ reactor and/or by decreasing the amount of hydroxyapatite in composites.

**Mechanical properties**

The mechanical properties of SPU's and HA/SPUs composites obtained with either glutamine or ascorbic acid as chain extenders showed an initial elastic behavior, although after the yield point had an elastomeric behavior (see supplementary data S2). In general, it was observed that unfilled polymer behave like elastomers although the addition of HA reduced the strain being the effect was more marked when the addition was done by mechanical mixing. In terms of tensile strength there was also a reduction when the HA was added to the ascorbic acid based polyurethanes being more severe by HA addition by physical mixing as the incorporation of the HA creates stress concentrators which cause the strength to decrease. In the same manner, SPUGL composites showed a mechanical

Journal of Materials Chemistry B Accepted Manuscript

reduction in terms of tensile strength when HA was added. Even when a good HA dispersion was not observed by SEM on these composites their higher molecular weight can be responsible for this behaviour (see Table 2). The higher strength of the neat polyurethane is attributable to the segmented nature of the polymers, hydrogen bonding of the urethane groups in addition to the urea links formed. The mechanical behaviour of these materials can also be attributed mainly to the PCL in the SPU matrix as once unfolded and aligned, the PCL chains tend to sustain the load and to show an increase in resistance. Table 2 summarizes de tensile mechanical properties of HA/SPU composites prepared during the course of this study. In general, it was observed that composites prepared with ascorbic acid as chain extender yielded higher Young´s modulus and tensile strength than composites prepared with glutamine when HA was incorporated during prepolymer formation.

The molecular weight of the SPU´s was sufficiently high to impart high strength and elasticity to the films prepared by solvent evaporation. However, for SPUM presented a bimodal distribution with an oligomer fraction resulting in a non-elastomeric fragile material. Low molecular weight in SPUAA can also be explained by the presence of moisture during reactions. Water will compete with the diisocyanate in undesirable side reactions such as urea-forming and Biuret or allophanate formation thus limiting the polymer molecular weight [25-26].

**Characterization of degraded SPU**

Accelerated degradations were carried out in extreme conditions of temperature and pH in order to understand behaviour SPU degradation as it will face both hydrolytic and oxidative environments within the body. As shown in Table 3, acidic hydrolysis led to a higher mass

Journal of Materials Chemistry B Accepted Manuscript

loss, followed by alkaline hydrolysis, due to the high proportion of PCL in the segmented poly (urethane ureas) i.e. 74% calculated from stoichiometry. The oxidative degradation showed lower mass loss compared to acidic and alkaline hydrolytic degradation but higher than distilled boiling water as previously reported [27].

Table 3 also shows the SPU mass loss during incubation in PBS solution. Mass loss was lower in SPUAA and SPUGL than in SPUAA-I and SPUGL-I being these values similar to those obtained during their accelerated degradation in distilled water.

### *In vitro* cytocompatibility studies

#### *MTT* tests

Cell viability of the SPUAA, SPUAA-I and SPUGL-I 24 h extracts exposed both at 24 h and 72 h showed little difference compared to the negative controls (cells only); all displaying relatively similar levels of mitochondrial activity (Figure 6). Only SPUGL 24 h eluants caused a drop in cell viability (less than 80%) when exposed for a period of 72 h. 48 h eluants from SPUAA exposed for 24 h and 72 h and 48 h SPUGL eluants exposed during 72 h showed higher toxicity. However, it should be noted that when 20 wt% HA was incorporated during prepolymer formation, osteoblast viability was recovered (from 60% up to 80%).

### Cell proliferation studies

Figure 7 shows that cell proliferation was maintained up to 14 days for SPUAA although it was higher at day 3 and then decreased. When HA was added during prepolymer formation (SPUAA-I), however, proliferation decreased at all four time points. For SPUGL and SPUGL-I cell proliferation was less than 20%, being the lowest of all conditions studied.

Journal of Materials Chemistry B Accepted Manuscript

## 4 DISCUSSION

### Physicochemical and mechanical properties of HA-SPU composites

There is a continuing interest in designing new polyurethanes from aliphatic diisocyanates, biodegradable polyols and biologically active chain extenders that can be used to repair or, preferably, regenerate damaged tissues including bone [14, 21, 24, 28]. Bearing this in mind, we synthesized segmented polyurethanes with polycaprolactone diol, HMDI and either L-glutamine or ascorbic acid as osteogenic chain extenders. Furthermore, two types of osteoconductive composites were prepared by adding HA during prepolymer synthesis or by mechanical mixing of the ceramic and the preformed polymer [29, 30, 31].

FTIR and $^{1}$H NMR showed evidence of polyurethane formation while solubility tests (in deuterated chloroform or DMF) suggested that linear or slightly crosslinked polymers (especially when ascorbic acid was used) were obtained in spite of the multifunctional nature of the chain extender. This can be attributed to the lower reactivity of carboxylic acids and secondary hydroxyl groups in glutamine and ascorbic acid respectively [31,32].

HA incorporation did not modify their FTIR spectra as expected due to its function as a filler and its incorporation in the matrix was evident from absorptions peaks corresponding to calcium phosphates even when the volume fraction was a low (ca. 6%.) [9,14,31-32].

DSC and XRD showed that semicrystalline polyurethanes were obtained where crystallinity tended to decrease by HA addition by mechanical mixing. It is possible that hydrogen bonds were formed between PCL chains and HA competed with intermolecular PCL hydrogen bonds. This was not observed when HA was incorporated during prepolymer formation as the -OH from HA may react with the isocyanate [14]. However, in both

Journal of Materials Chemistry B Accepted Manuscript

methods of HA addition, the XRD pattern of the composites showed that the crystal structure of HA remained unchanged.

In the temperature interval of our dynamic mechanic experiments the poly (ε-caprolactone) shows only the main relaxation associated with the co-operative rearrangements of the polymer chain in the amorphous phase. However, the $T_g$ of the PCL tend to increase due to chain movement restrictions, on one hand due to the presence of the rigid segments (approximately 25%) and on the other hand due to the presence of a rigid HA filler. From these competing factors, the rigid segment content predominates as the shift in $T_g$ was higher on the neat polymers.

The effect of HA inclusions can also be discussed in terms of their mechanical properties. Incorporation of HA had a reinforcing effect only when the filler was incorporated by mixing (SPUAA-M and SPUGL-M) i.e. an increase in modulus, which may originate from stiffer HA particles and the interaction between the HA and matrix. Similar reports suggest that there is a reinforcing effect up to 50 wt.% of HA in the compressive strength [14]. In agreement with these observations, $T_g$ increased rendering composites with higher modulus. Improvement of thermal stability can also be attributed to the hydrogen bond formation between the urethane group (NHCOO) of SPU and OH from HA. The mechanical properties of the SPU are also influenced by their molecular weight which in this case, is lower than the reported previously for other PCL-based (polyurethanes) [33]. Despite of this, the molecular weight achieved was high enough for film formation and mechanical testing [22]. Therefore, composites prepared by adding HA during prepolymer formation exhibited the highest molecular weight and the best mechanical performance in terms of Young´s modulus and tensile strength. These values are still far from the cortical bone (12-18 GPa) and spongy bone (100-500 MPa). Furthermore, the mechanical

Journal of Materials Chemistry B Accepted Manuscript

properties achieved are still low when compared to foamed polyurethanes used to mimic trabecular bone [33-34]. The reduction in mechanical properties can be also attributed to a poor dispersion as shown by SEM as tensile test are highly sensitive to agglomeration and defects in the specimen. In general HA particles will be easily attracted together due to their very high surface area and high surface energy [15]

A way to compensate for the low mechanical properties achieved by these composites is by producing an osteoconductive porous structure that would enhance the migration of osteoblasts from surrounding bone into the porous scaffold and accelerate the bone regeneration process. It is generally accepted that the presence of interconnected pores will allow the transportation of nutrients to cells and that it will enhance capillary formation as the polymer is being biodegraded [32-33]. Although compressive mechanical properties were not measured in the porous scaffolds prepared by supercritical $CO_2$, the presence of HA in the composite foam is expected to benefit their mechanical behavior as reported by others [14].

Biodegradation is another important factor when designing biomaterials for tissue engineering applications. Biodegradability of the new SPU and HA/SPU composites in PBS was slow during 6 months as shown in Table 3. However, this behavior can be modulated by two factors. On one hand, it will be accelerated by water absorption and on the other hand, can be accelerated *in vivo* by the presence of acids, oxidants or specific enzymes. In the first case, it was observed that the presence of hydroxyapatite increased fluid absorption in PBS 11.04±0.32% and 9.11±0.43% for SPUA and SPUG respectively vs. 16.30 ±0.23% and 18.25± 0.34% for SPUA-I and SPUG-I respectively). Regarding their chemical accelerated degradation, it was found that the synthesized SPU and HA/SPU composites can be extensively degraded both by changes in the pH and by oxidizing

Journal of Materials Chemistry B Accepted Manuscript

conditions in spite of being slightly hydrophilic. Changes in pH will degrade mainly the PCL soft segment due to ester hydrolysis. Hydrolysis of PCL at high pH, although unlikely *in vivo*, has further advantages as it introduces -OH and -COOH groups on the surface, rendering hydrophilic substrates that will increase its degradation. Under normal conditions polyester based SPU´s tend to be stable towards oxidation; however metabolic products such as peroxides secreted by macrophages are strong oxidants that greatly accelerate the degradation process [36].

**Biological performance of HA-SPU composites**

Cytotoxicity was reduced in the composites compared to neat polyurethanes, especially those containing ascorbic acid as chain extender as observed from MTT test results. Cell proliferation, although less than 80%, was higher in polyurethanes containing ascorbic acid, followed by those HA composites prepared *in situ*. Therefore, it seems that this osteogenic compound, even when it was used in higher amounts (approximately 3 wt. % of ascorbic acid for SPU synthesis) than the recommended for osteoblast cell culture (50 μg/ml or 150 mg/l) [34] is more important than the presence of an osteoconductor. In low concentrations, ascorbic acid or L-ascorbate-2-phosphate, a long acting ascorbic acid derivative, is essential for the expression of osteoblastic markers (collagen synthesis and alkaline phosphatase activity) and mineralization [37]. However, at physiological pH is in the ascorbate form it is quite unstable (it readily oxidizes to dehydro-ascorbate) therefore, it is possible that a continuous release from the SPU was beneficial. Glutamine containing polyurethanes exhibited poor cell proliferation probably because of the presence of a-non-alkaline nitrogen, as an amide is the functional group in the lateral chain. We have reported that a better cell response is achieved by the presence of alkaline nitrogen atoms in the lateral

Journal of Materials Chemistry B Accepted Manuscript

chain i.e. when arginine as chain extenders [27]. In addition, the concentration of L-glutamine found in culture media range from 2 to 4 mM which is also lower than the amount used in this study. Furthermore, L-glutamine can be degraded to ammonia with time and temperature (from example from 8 mM up to 3 mM after 7 days at 37°C), explaining the poor cell proliferation.

The properties exhibited by SPUAA and SPUGL and their HA composites were not comparable to other polyurethanes suggested for bone tissue regeneration [11-17] in terms of mechanical properties and injectability. However, the synthesis of segmented polyurethanes using osteogenic compounds as chain extenders was demonstrated and the results are in agreement with reports that used copolymers of ascorbic acid, lysine diisocyanate and glycerol [19]. A further advantage of the polyurethanes reported here is that an osteoconductive ceramic can be incorporated during polymer synthesis and that porosity can be introduced by a clean technology (supercritical $CO_2$) after the synthesis of their HA composite.

Taking all this into account, the reduction in the concentration of these osteogenic compounds and studies regarding their release as well as *in vivo* studies is recommended for future polyurethane synthesis and their potential use in bone tissue regeneration.

## 5 CONCLUSIONS

SPU's prepared with either L-glutamine or ascorbic acid as chain extenders were semicrystalline polymers with low $T_g$ and elastomeric behavior. They exhibited a molecular weight high enough for film forming behave as thermoplastic polymers as they were soluble in THF, chloroform and DMF and preferably degraded under alkaline and acidic conditions. Composites prepared with 20 wt.% of HA added after polymer formation

Journal of Materials Chemistry B Accepted Manuscript

improved their tensile modulus. Composites prepared with ascorbic acid as chain extender and HA added during prepolymer formation (SPUAA-I) are good candidates for bone tissue regeneration as the 24 h eluants to which cells were exposed for 24 h and 72 h were not cytotoxic to human alveolar osteoblast and allowed their proliferation up to 14 days. This performance, however, can be improved by using supercritical $CO_2$ that proved to be useful to shape them into porous biodegradable polyurethanes.

Acknowledgments. This work was supported by CONACYT (Mexico) Grants 96865/96865. XRD measurements were performed at LANNBIO Cinvestav Mérida, under support from projects FOMIX-Yucatán 2008-108160 and CONACYT LAB-2009-01 No. 123913. Technical help is acknowledged to MSc. Daniel Aguilar.

## REFERENCES

1. L. Zhou, L. Yu, M. Ding, J. Li, H. Tan, Z. Wang and Q. Fu, *Macromolecules*, 2011, **44**, 857-864.
2. E. Jimi, S. Hirata, K. Osawa, M. Terashita, C. Kitamura and H. Fukushima, *Int J Dent*, **2012**, 1-7.
3. L. Yuchun, L. Jing and T. Swee-Hin, *Biotech Adv*, 2012, **31**, 688-705.
4. S. Gogolewski, *Colloid Polym Sci*, 1989, **267**, 757-785.
5. L. S. Nair and C. T. Laurencin, *Prog Polym Sci*, 2007, **32**, 762-798
6. J. C. Middleton and A. J. Tipton, *Biomaterials*, 2000, **21**, 2335-2346.
7. A. J. Salgado, O. P. Coutinho and R. L. Reis, *Macromol Biosci*, 2004, **4**, 743-765.
8. C. Boissard, P.-E. Bourban, A. Tami, M. Alini and D. Eglin, *Acta Biomater*, 2009, **5**, 3316-3327.
9. L. Wang, Y. Li, Y. Zuo, L. Zhang, Q. Zou, L. Cheng and H. Jiang, *Biomed Mater*, 2009, **4**,2, 025003.
10. J. L. Ryszkowska, M. Auguścik, A. Sheikh and A. R. Boccaccini, *Compos Sci Technol*, 2010, **70**, 1894-1908.
11. K. D. Kavlock; T. W. Pechar; J. O. Hollinger; S. A. Guelcher;A. S. Goldstein, *Acta Biomater* 2007, **3**, 475-484
12. I. C. Bonzani, R. Adhikari, S. Houshyar, R. Mayadunne, P. Gunatillake and M. M. Stevens, *Biomaterials*, 2007, **28**, 423-433.

Journal of Materials Chemistry B Accepted Manuscript

13. R. Adhikari, P. A. Gunatillake, I. Griffiths, L. Tatai, M. Wickramaratna, S. Houshyar, T. Moore, R. Mayadunne, J. Field and M. McGee, *Biomaterials*, 2008, **29**, 3762-3770.
14. H. Liu, L. Zhang, Y. Zuo, L. Wang, D. Huang, J. Shen, P. Shi and Y. Li, *J Appl Polym Sci*, 2009, **112**, 2968-2975.
15. H. Liu, L. Zhang, J. Li, Q. Zou, Y. Zuo, W. Tian and Y. Li, *J Biomat Sci: Polym Ed*, 2010, **21**, 1619-1636.
16. A. a. R. De Oliveira; S. M. De Carvalho; M. De Fátima Leite; R. L. Oréfice;M. De Magalhães Pereira, *J Biomed Mater Res Part B: Appl Biomater*, 2012,**100B**, 1387-1396.
17. M. Bil, J. Ryszkowska, P. Woźniak, K. J. Kurzydłowski and M. Lewandowska-Szumieł, *Acta Biomater*, 2010, **6**, 2501-2510.
18. M. C. T. Cabral, M. A. Costa and M. H. Fernandes, *J Mater Sci: Mater Med*, 2007, **18**, 1079-1088.
19. J. Zhang; B. A. Doll; E. J. Beckman; J. O. Hollinger, *J Biomed Mater Res* Part A, 2003, **67A**, 389-400
20. S. Jiang, Ji, Xiangling,An, Lijia,Jiang, Bingzheng, *Polymer*, 2001, **42**, 3901-3907.
21. S. A. Guelcher; K. M. Gallagher; J. E. Didier; D. B. Klinedinst; J. S. Doctor; A. S. Goldstein; G. L. Wilkes; E. J. Beckman;J. O. Hollinger, *Acta Biomater* 2005, **1**, 471-484.
22. B. Bogdanov, V. Toncheva, E. Schacht, L. Finelli, B. Sarti and M. Scandola, *Polymer*, 1999, **40**, 3171-3182.
23. K. Gorna, S. Polowinski and S. Gogolewski, *J Polym Sci Part A: Polym Chem*, 2002, **40**, 156-170.
24. L. May-Hernández; F. Hernández-Sánchez; J. Gomez-Ribelles; R. Sabater, I Serra. *J Appl Polym Sci*, 2011, **119**, 2093-2104.
25. M. K. Hassan, K. A. Mauritz, R. F. Storey, J. S. Wiggins, *J Polym Sci Part A: Polym Chem*, 2006, **44**, 2990-3000.
26. G. Skarja and K. Woodhouse, *J Biomater Sci: Polym Ed*, 1998, **9**, 271-295.
27. L.H. Chan-Chan, C. Tkaczyk, R.F Vargas Coronado, J.M. Cervanes Uc, M. Tabriziam, J.V Cauich Rodríguez, *J Mater Sci: Mater Med*, 2013, **4**, 4928-4931.
28. A. Marcos-Fernández, G. A. Abraham, J. Valentín and J. S. Román, *Polymer*, 2006, **47**, 785-798
29. C. Boissard; P.-E. Bourban; A. Tami; M. Alini; D. Eglin, *Acta Biomater*, 2009, **5**, 3316-3327
30. Z. Dong, Y. Li and Q. Zou, *Appl Surf Sci*, 2009, **255**, 6087-6091.
31. A. Martínez-Valencia, G. Carbajal-De la Torre, R. Torres-Sánchez, L. Téllez-Jurado, H. Esparza-Ponce, *Int. J. Phys. Sci*, 2011, **6**, 2731-2743.
32. G. Tripathi; B. Basu, A, *Ceram Int*, 2012, **38**, 341-349
33. A.Asefnejad, A.Behnamghader, M.T.Khorasani and B. Farsadzadeh, *Int J Nanomed*, 2011, **6**, 93-100.
34. J.A Roeter, S Deb; *J Mater Sci: Mater Med*, 2004, **15**, 413-418.

Journal of Materials Chemistry B Accepted Manuscript

35. H. J. Griesser, *Polym Degrad Stab* 1991, **33**, 329-354
36. Y. Feng, Li, Chongyang, *Polym Degrad Stab*, 2006, **91**, 1711-1716
37. S. Deb; R. Mandegaran; L. Di Silvio, *J Mater Sci: Mater Med*, 2010, **21**, 893-905

Journal of Materials Chemistry B Accepted Manuscript

**FIGURE CAPTIONS**

Scheme 1. Suggested mechanism for the synthesis the poly (urethane-urea) prepared with either glutamine (GL) or ascorbic acid (AA). A) Formation of linear polymers and B) formation of crosslinked polymers

Figure 1. $^{1}$H NMR spectra of the SPUAA and SPUGL

Figure 2. FTIR spectra of the SPUAA (a) and SPUGL (b) with 20 wt.% HA. Hydroxyapatite was incorporated by mechanical mixing (SPUM) or during prepolymer formation (SPUI).

Figure 3. XRD patterns of SPUAA (a) and SPUGL (b) with 20 wt. % HA. Hydroxyapatite was incorporated by mechanical mixing (SPUM) or during prepolymer formation (SPUI).

Figure 4. SEM images of dense HA/SPU composites synthesized using ascorbic acid (left) and glutamine (right) as chain extenders. Unfilled SPU (a, b), HA incorporated during polymerization (c, d) and HA incorporated by physical mixing (e, f).

Figure 5. SEM images of HA/SPU composites after supercritical $CO_2$ treatment a) SPUAA-I/HA HT 30 min DT 15 min. b) SPUAA-I/HA HT 2 h DT 1 hr. C) SPUGL-I/HA HT 30 min DT 15 min, d) SPUGL-I/HA HT 2h DT 1 h.

Figure 6. Osteoblast cell viability of SPU extracts by MTT assay.

Figure 7. Cell proliferation on SPU´s by Alamar blue$^{TM}$ assay.

Journal of Materials Chemistry B Accepted Manuscript

**Table 1.- Thermal Properties of the Segmented Polyurethanes**

| Polyurethane Type | DSC [a] | | | DMA [b] | TGA [c] |
|---|---|---|---|---|---|
| | $T_m$ (°C) | ΔH (J/g) | Crystallinity % | $T_g$ (°C) | $T_d$ (°C) |
| Pristine PCL 2000 | 50.3 | 21.9 | 40 | ---------- | ---------- |
| SPUAA | 53.8 | 39.2 | 38 | -25.0 | 396.2 |
| SPUAA-I | 53.7 | 33.3 | 41 | -33.8 | 427.6 |
| SPUAA-M | 50.3 | 31.8 | 31 | -36.0 | 417.7 |
| SPUGL | 51.4 | 41.7 | 40 | -25.1 | 397.2 |
| SPUGL-I | 50.6 | 32.1 | 41 | -24.5 | 432.8 |
| SPUGL-M | 54.0 | 43.9 | 39 | -38.1 | 412.0 |

[a] Obtained by DSC (first heating) [18]
[b] Temperature at the maximum of the loss tangent in the main relaxation process by DMA
[c] Temperature at the maximum rate of the decomposition by TGA

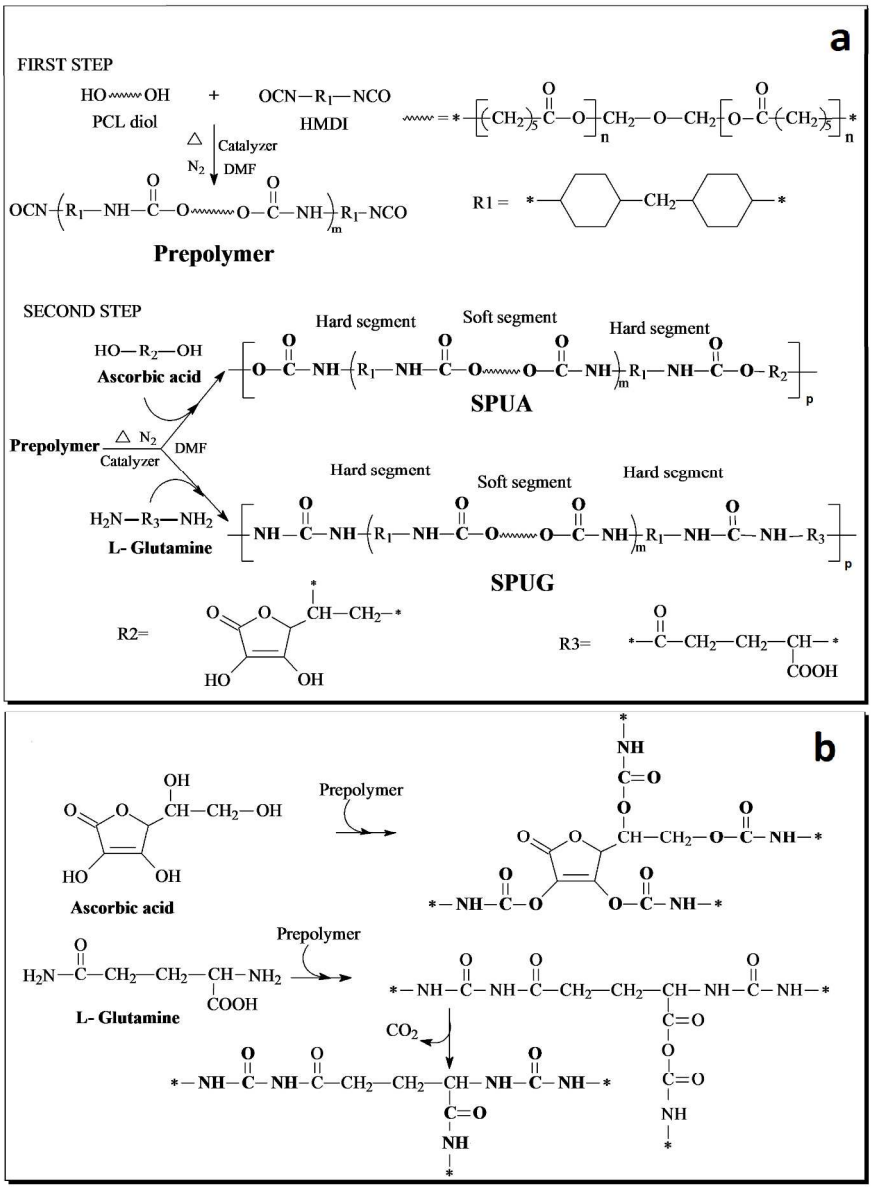
**Table 2.- Mechanical properties of HA/SPU composites**

| Polyurethane type | Mechanical properties | | | Molecular weight | | |
|---|---|---|---|---|---|---|
| | σ (MPa) | ε (%) | E (MPa) | $M_n$ | $M_w$ | $M_w/M_n$ |
| SPUAA | 47.75 ± 16.7 | 1298 ± 285.6 | 26.77 ± 25.6 | 28,885 | 53,679 | 1.85 |
| SPUAA-I | 24.50 ± 3.62 | 1634 ± 165 | 19.0 ± 2.17 | 30,065 | 82,631 | 2.74 |
| SPUAA-M | 9.18 ± 0.56 | 27.1 ± 10.13 | 39.7 ± 8.20 | 19,578 | 58,456 | 2.98 |
| SPU GL | 19.66 ± 11.23 | 1879 ± 302.4 | 12.0 ± 2.26 | 75,490 | 153,540 | 2.03 |
| SPUGL- I | 14.44 ± 1.81 | 1561 ± 261.9 | 11.61 ± 1.41 | 177,460 | 225,850 | 1.27 |
| SPUGL-M | 8.95 ± 1.69 | 39.04 ± 14.73 | 36.77 ± 11.21 | 52,147 | 113,120 | 1.55 |

**Table 3.- Mass loss (%) of SPUs after degradation in different media**

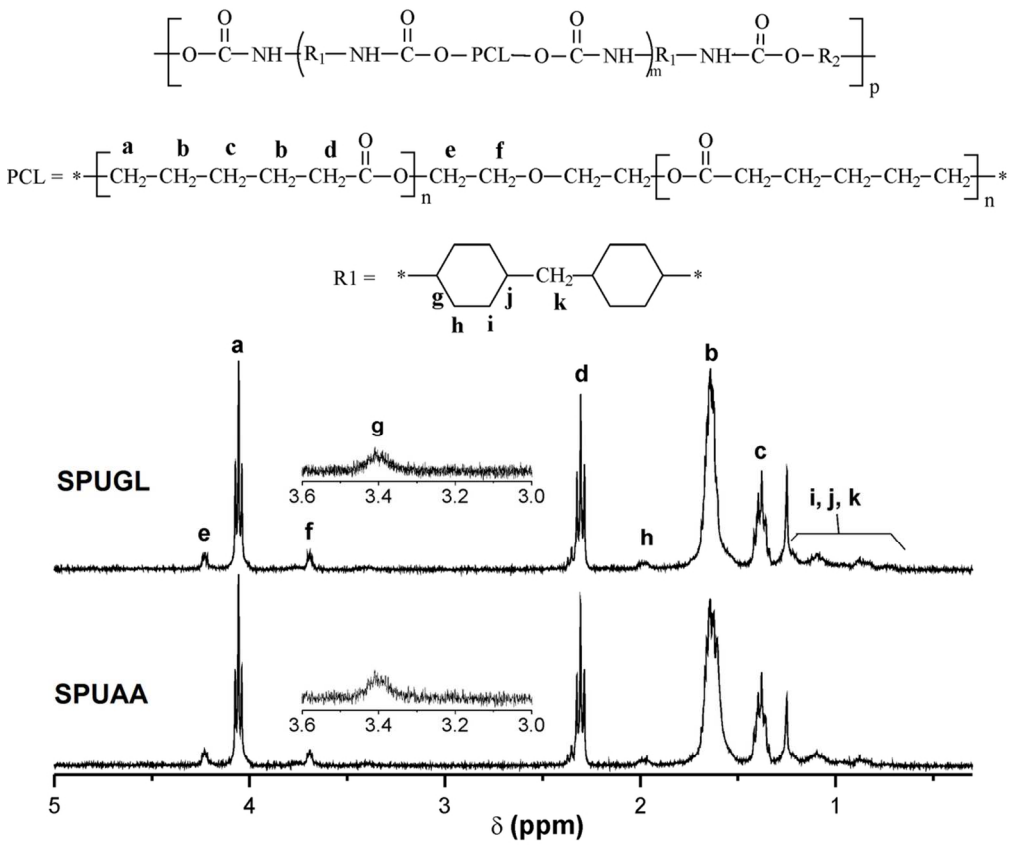
| Polyurethane type | Hydrolytic degradation | | | Oxidative degradation | PBS Degradation |
|---|---|---|---|---|---|
| | Water | HCL (2 N) | NaOH (5 M) | $H_2O_2$ (30 vol. %) | 120 days |
| SPUAA | 1.34 ± 1.12 | 84.41 ± 0.93 | 34.5 ± 4.05 | 25.84 ± 2.63 | 4.63 ± 0.16 |
| SPUAA-I | 3.52 ± 2.37 | 88.22 ± 2.43 | 46.3 ± 0.45 | 38.35 ± 0.11 | 5.8 ± 0.56 |
| SPUGL | 2.69 ± 1.80 | 82.60 ± 1.46 | 28.6 ± 0.66 | 26.58 ± 0.42 | 2.89 ± 0.73 |
| SPUGL-I | 5.61 ± 0.69 | 90.22 ± 3.36 | 50.2 ± 1.25 | 44.97 ± 0.22 | 5.5 ± 0.06 |

Journal of Materials Chemistry B Accepted Manuscript



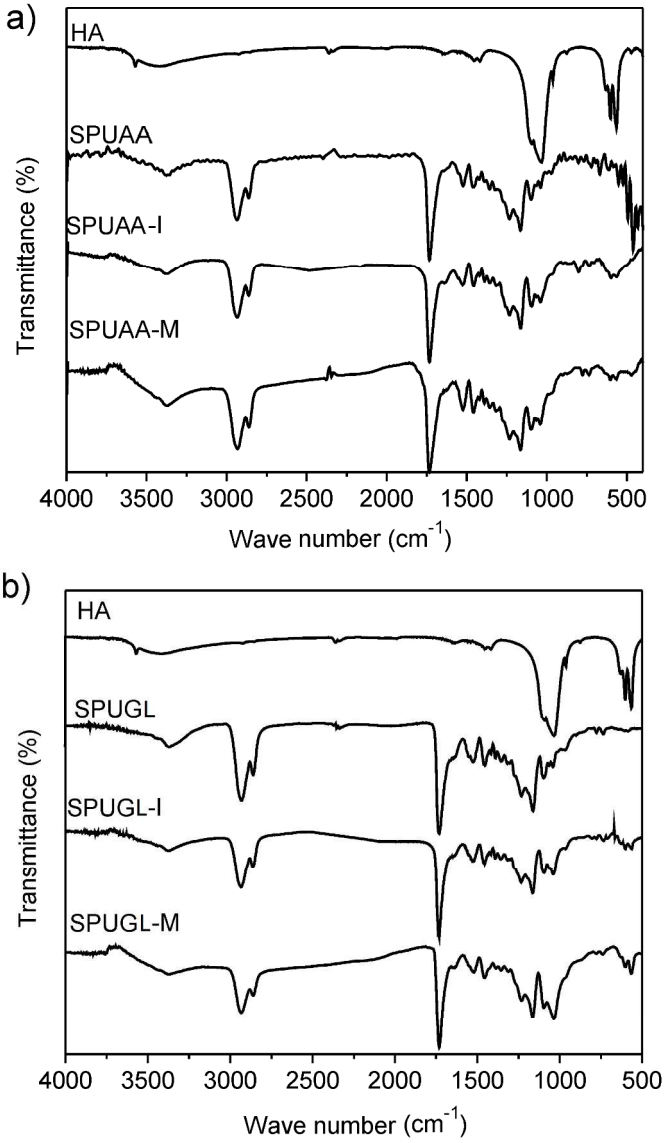

Suggested mechanism for the synthesis the poly (urethane-urea) prepared with either glutamine (GL) or ascorbic acid (AA). A) Formation of linear polymers and B) formation of crosslinked polymers
656x890mm (96 x 96 DPI)

Journal of Materials Chemistry B Accepted Manuscript



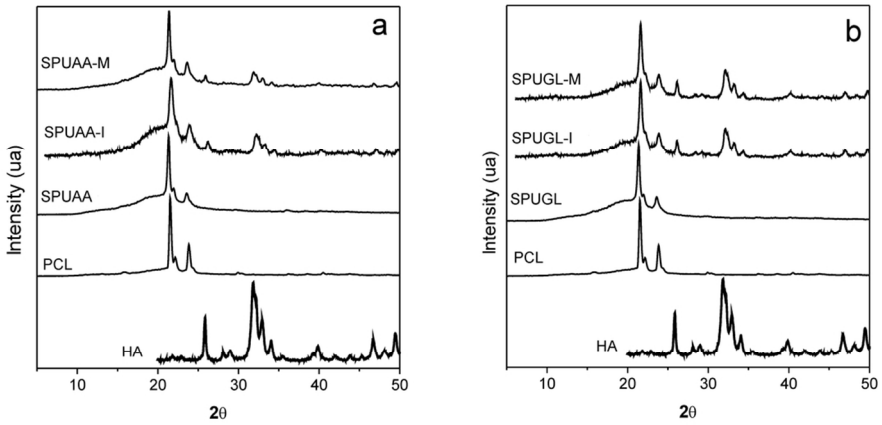

1H NMR spectra of the SPUAA and SPUGL
51x43mm (600 x 600 DPI)

Journal of Materials Chemistry B Accepted Manuscript



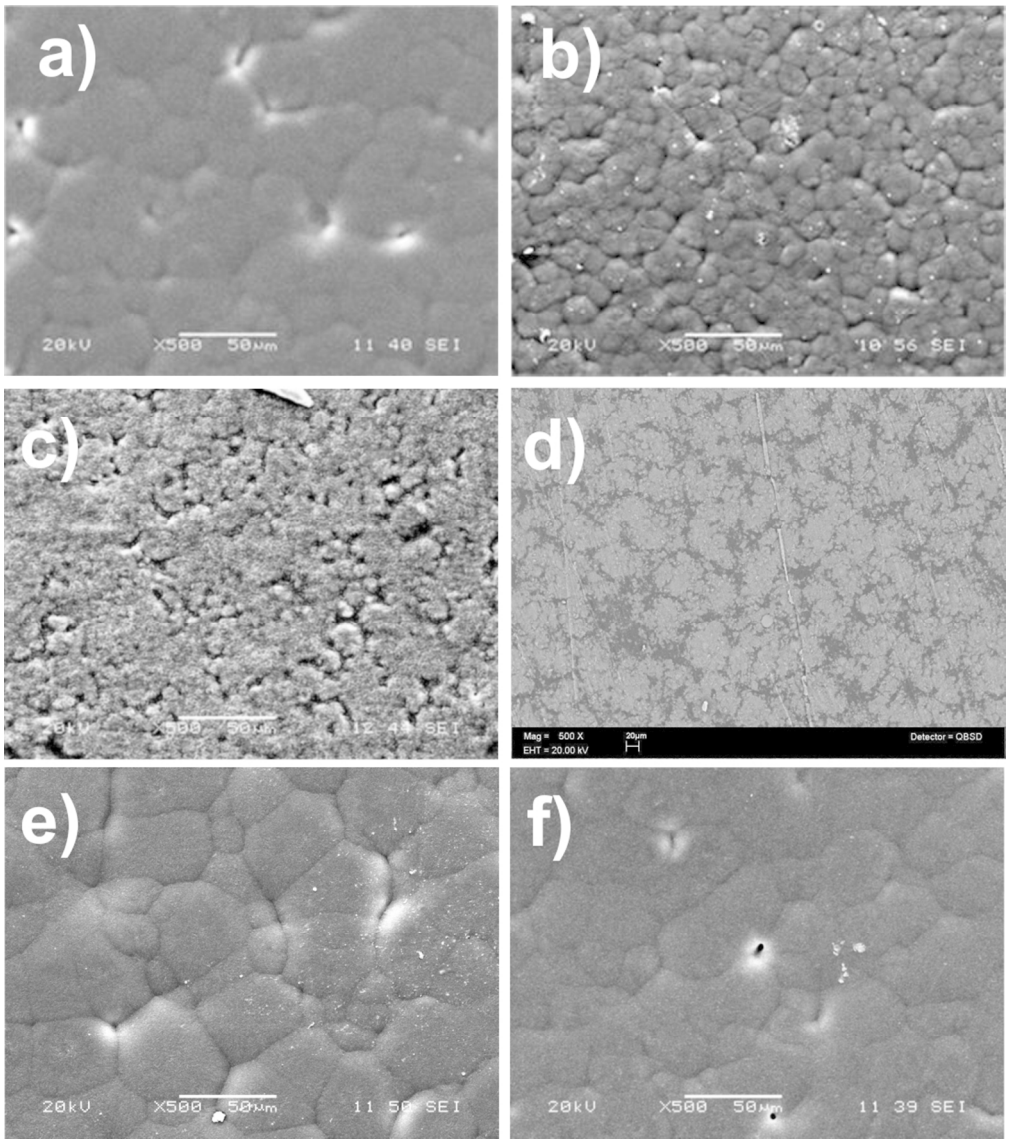

FTIR spectra of the SPUAA (a) and SPUGL (b) with 20 wt. % HA. Hydroxyapatite was incorporated by mechanical mixing (SPU-M) or during prepolymer formation (SPU-I).
565x963mm (96 x 96 DPI)

Journal of Materials Chemistry B Accepted Manuscript



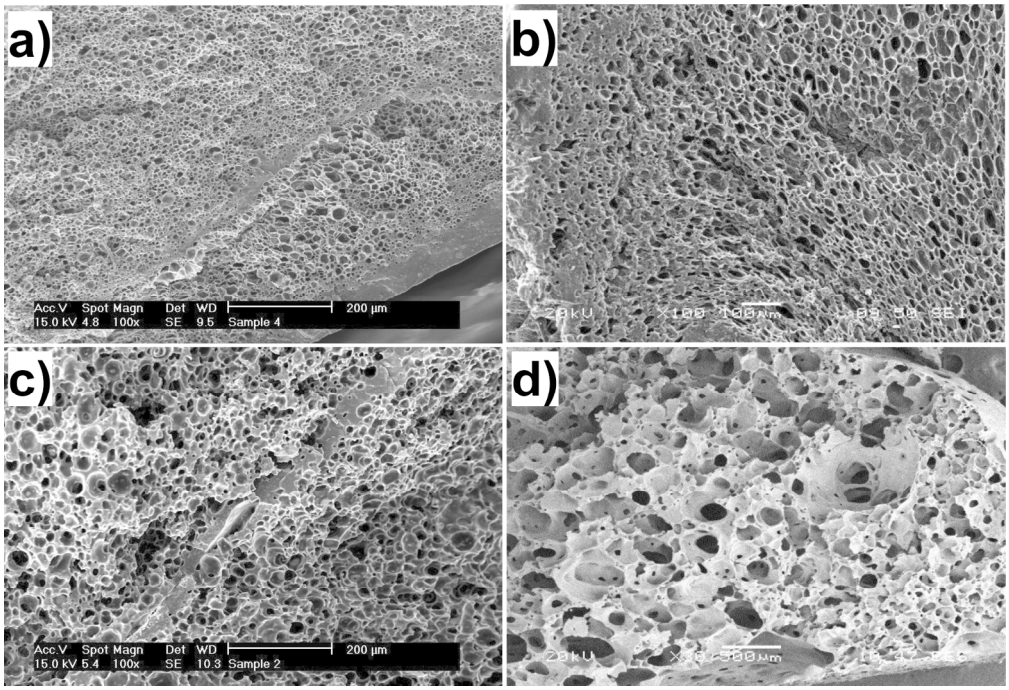

XRD patterns of SPUAA (a) and SPUGL (b) with 20 wt. % HA. Hydroxyapatite was incorporated by mechanical mixing (SPU-M) or during prepolymer formation (SPU-I).
103x52mm (300 x 300 DPI)

Journal of Materials Chemistry B Accepted Manuscript



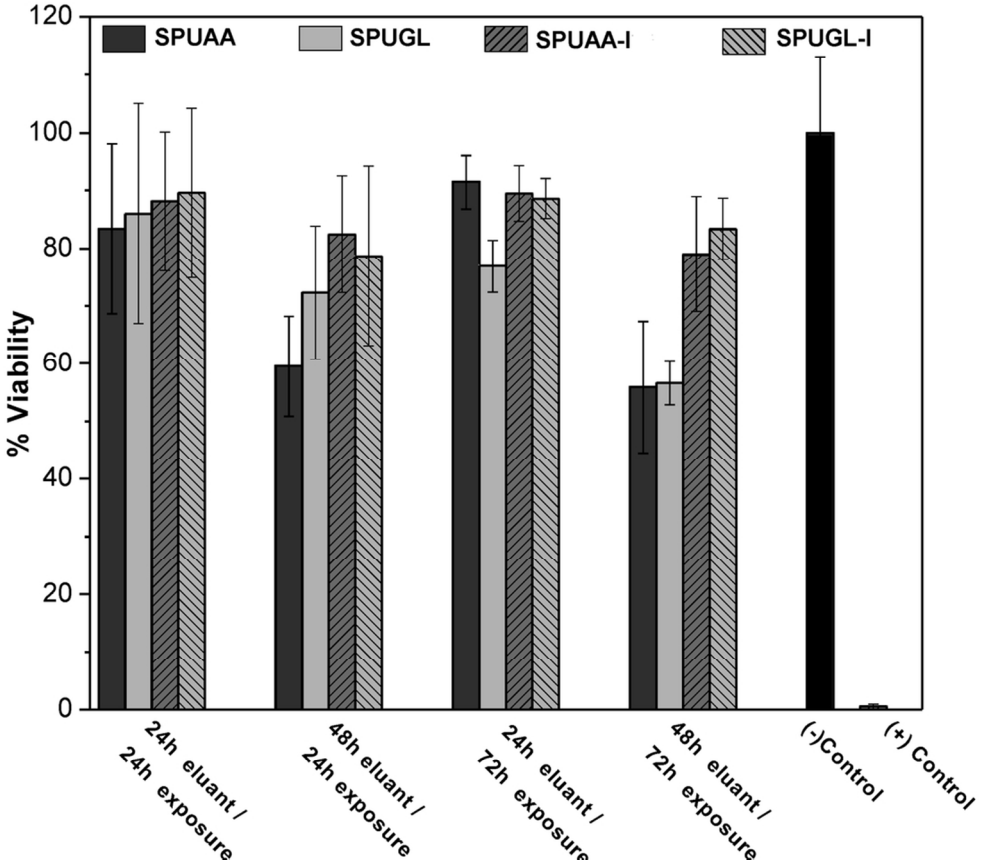

. SEM images of dense HA/SPU composites synthesized using ascorbic acid (left) and glutamine (right) as chain extenders. Unfilled SPU (a, b), HA incorporated during polymerization (c, d) and HA incorporated by physical mixing (e, f).
92x104mm (300 x 300 DPI)

Journal of Materials Chemistry B Accepted Manuscript



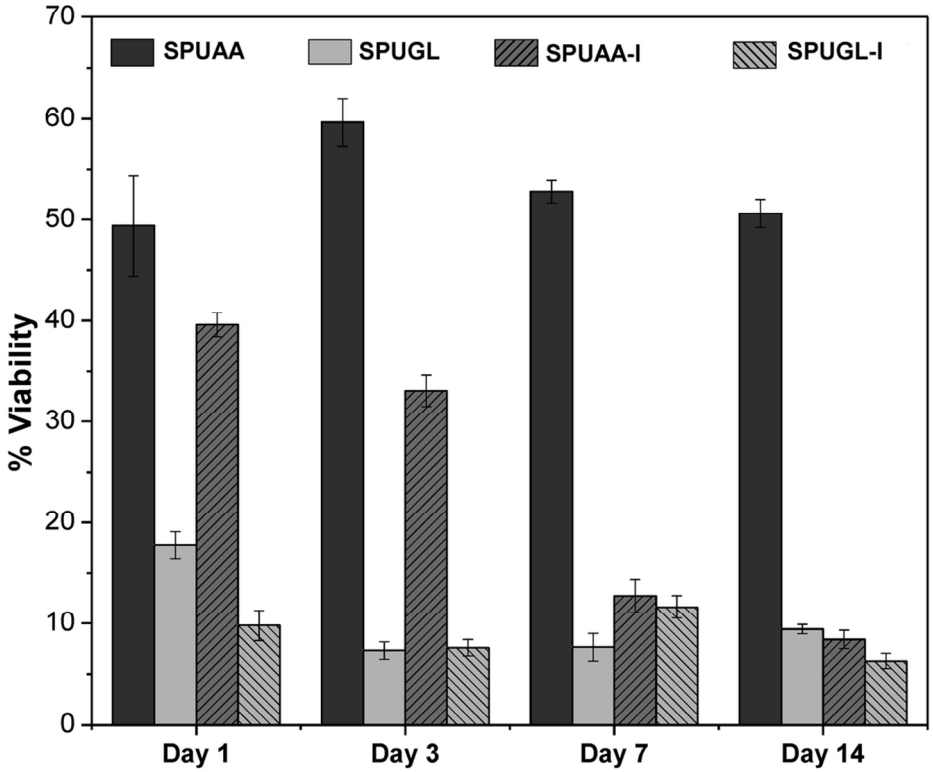

SEM images of HA/SPU composites after supercritical CO2 treatment a) SPUAA-I/HA HT 30 min DT 15 min. b) SPUAA-I/HA HT 2 h DT 1 hr. C) SPUGL-I/HA HT 30 min DT 15 min, d) SPUGL-I/HA HT 2h DT 1 h.
170x116mm (300 x 300 DPI)

Journal of Materials Chemistry B Accepted Manuscript




Osteoblast cell viability of SPU extracts by MTT assay
94x85mm (300 x 300 DPI)

Journal of Materials Chemistry B Accepted Manuscript




Cell proliferation on SPU´s by Alamar blueTM assay.
103x92mm (300 x 300 DPI)

Journal of Materials Chemistry B Accepted Manuscript