Poly[octanediol-co-(citric acid)-co-(sebacic acid)] elastomers: novel bio-elastomers for tissue engineering

Ivan Djordjevic,† Namita Roy Choudhury, Naba K Dutta and Sunil Kumar∗

Abstract
This review focuses on a new class of elastomers, namely poly{octanediol-co-(citric acid)-co-(sebacic acid)} (pOCS), synthesised from 1,8-octanediol, citric acid and sebacic acid in a catalyst-free polyesterification reaction. The review begins with a detailed description of the synthesis, characterisation and structure–property–performance relationship of some reported elastomers suitable for tissue engineering. The control of the physicochemical properties of the new pOCS by simple variation of initial monomer concentrations in polyesterification forms the pivotal part of the synthesis. As tissue engineering requires complex designs, thin films and porous three-dimensional structures of pOCS were fabricated to demonstrate their ease of processing. The fundamental material properties of pOCS are discussed for pOCS pre-polymers and final polymers. The elastomers exhibit versatility in mechanical properties, hydration and hydrolytic degradation, as determined by their chemical structure. Surface analysis of spin-coated pOCS suggests that the surface morphology, chemistry and concentration of the surface functional groups can be controlled simply by varying the initial citric acid/sebacic acid concentration in polyesterification. These tunable molecular architectures and material properties are crucial in biological interactions. The in vitro biocompatibility testing of pOCS with MG63 osteoblast-like cells suggests that pOCS is an excellent candidate for potential elastic biomaterials for tissue engineering applications without the need for any post-synthesis modification.

Keywords: citrate/sebacate polyesters; elastomers; tissue engineering; scaffold materials

INTRODUCTION
Over the last two decades, tissue engineering has steadily developed and emerged as a new multidisciplinary research field in regenerative medicine. The main strategy involves tissue regeneration by basic, tissue-specific cells that are seeded into specially designed synthetic matrices called scaffolds. The main guiding principle in scaffold development is that the scaffolding material should resemble the natural extracellular matrix (ECM) of the target tissue. The ECM of most tissues is an elastic, resilient and highly hydrated polymer network. The natural ECM is produced by the proliferating tissue-specific cells and it plays a major role in intercellular communication and provides physical support for cells in functional tissues. The role of synthetic scaffolds in the engineering of specific tissues is to mimic natural conditions of ECM in order to guide cellular migration and proliferation during the initial stages of tissue regeneration. Ideally, the synthetic scaffold should biologically degrade over time leaving a healthy, functional engineered tissue. In terms of scaffold implantation, the applied synthetic material should exhibit minimal immunological response from the surrounding metabolism. Due to the high sensitivity of material–body interactions, careful design and tailoring of scaffold materials play a crucial role in successful tissue engineering.

Elastomeric polyesters based on citric acid (CA) and sebacic acid (SA) have recently been presented as a new generation of synthetic scaffolding biomaterials. These materials can be synthesised economically from non-toxic monomers (CA and SA) using basic chemical procedures. Both CA and SA molecules are part of different metabolic cycles in the human body and, therefore, are considered ‘biocompatible’. When these multifunctional acids (CA and SA) react with multifunctional alcohols in catalyst-free polyesterification (polycondensation) reactions, they yield hydrophilic polyesters with high degrees of elasticity. Table 1 shows and compares the mechanical and degradation properties of some recently developed synthetic elastomers, i.e. poly(octanediol citrate) (POC) and poly(glycerol sebacate) (PGS), with some relevant human tissues and some of the most commonly used US Food and Drug Administration (FDA)-approved synthetic tissue engineering materials, i.e. poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymer poly[(lactic acid)-co-(glycolic acid)] (PLGA). Both PLA and PGA, together with their copolymer PLGA, have been used clinically over a very long period providing substantial data regarding their performance as biomaterials. However, these materials are relatively rigid with elastic moduli values in excess of 1000 MPa, which limits their application in soft tissue engineering (Table 1). At higher elon-

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Associate Professor Sunil Kumar’s current research is centred on improving the fixation of orthopaedic implants through surface coating and plasma processing, including the synthesis and surface engineering of polymeric and ceramic tissue engineering scaffolds. This research has led to three recent patent applications. He currently peer-reviews for over 25 international research journals and funding agencies.

Ivan Djordjevic is a research associate at the Institute for Multidisciplinary Research, University of Belgrade. He completed his PhD in polyester elastomers aimed for tissue engineering applications at the Ian Wark Research Institute, University of South Australia. His current research interests are focused on development of novel tissue engineering systems.

Naba Dutta received his PhD in Polymer Science and Engineering from Indian Institute of Technology, India; followed by a post-doctoral position in CNRS, France. As an Associate Research Professor, at IWRI, University of South Australia his current research pertains to multi-component polymer/biopolymer systems with particular emphasis on the role of interface and its control.

Namita Roy Choudhury received her PhD in Polymer Science & Engineering from Indian Institute of Technology, India in 1990. She then completed a post-doctoral fellowship in CNRS, France and moved to University of South Australia in 1996. Prof. Choudhury’s research interest spans from Hybrid polymers to Biomimetic polymers and Tissue-Engineering Scaffolds.

Both PGS and POC are produced from trifunctional monomers (glycerol and CA) containing tertiary hydroxyl (–OH, glycerol) and tertiary carboxyl (–COOH, CA) groups (Fig. 1). It was reported that their tertiary functionalities mostly remain unreacted in polyesterification due to steric hindrance,46 which explains why PGS and POC polyester chains are assembled into linear structures with pendant –OH and –COOH functional groups.18,36 These functionalities play a major role in the formation of physical crosslinks between polymer chains, giving rise to the unique, rubber-like (elastomeric) morphology of both PGS and POC. The rubber-like morphology makes PGS and POC reminiscent of the biopolymers found in natural ECM such as collagen or elastin.18,31 Both the functionalities (–OH and –COOH) are most likely present on the materials’ surfaces making PGS and POC hydrophilic in contrast to hydrophobic PLA, PGA or PLGA, which often require additional surface treatments in order to impart hydrophilicity required for tissue engineering applications.18,31

The main advantage of CA- or SA-based polyester elastomers is that the basic bioresponsive properties such as mechanical properties can be tuned to mimic soft tissues more closely (Table 1). Apart from the elastic and low-modulus CA- and SA-based polyesters, elastomeric POC material has also found an application for bone tissue engineering. This elastomeric material has been mixed with the bone mineral hydroxyapatite (HA) in order to make it more rigid and compatible for bone cells (POC/HA; Table 1).34

The synthesis of both CA- and SA-based biocompatible polymers is realised by polycondensation, a well-defined and simple process of chemical condensation leading to the formation of a polymer by linking molecules of the monomers followed by release of water (or a similar simple substance). Figure 1 shows the chemical structures of PGS and POC polyester elastomers that can be processed into porous scaffolds for engineering of various tissues.
SA is a difunctional, linear carboxylic acid that naturally occurs in human metabolism. Polymers produced from SA and glycerol (PGS) have been approved for use in medical applications by the FDA. PGS or ‘biorubber’ material was designed to mimic the mechanical properties of ECM and has so far been utilised in the field of soft tissue engineering due to its ability to sustain and recover from deformation in mechanically dynamic environments. The reported work on PGS scaffolds and other synthetic elastomers containing SA has shown promising in vivo and in vitro compatibility with various biological systems such as blood and plasma, cell cultures of human foreskin fibroblasts, nerve cells and aortic endothelial smooth muscle cells. Furthermore, test results of immunological response to PGS implants showed minimal inflammation without granulation or formation of scar tissues after 60 days in vivo.

The immunological response is a sensitive process that follows the implantation of a foreign body such as a polymeric scaffold or any other biomaterial. The immediate foreign body response is related mainly to surface chemistry of biomaterials, but over longer periods of time, material degradation is equally important. A good correlation between long-term immunological reaction and in vivo degradation of PGS has been highlighted by several authors. A detailed study of the in vivo immunological response to PGS, in comparison with PLGA, was reported by Sundback et al. The results of this study demonstrated the similarity of early tissue response to PGS and PLGA over 7 days post-implantation. Significant increase of inflammatory responses was detected on PLGA after 21 and 35 days in vivo while the inflammatory response to PGS continued to decrease during the 60 day experiment. It was reported that the rapid increase in PLGA degradation after 20 days was the most likely cause of the enhanced inflammatory response. A reason for such an interpretation is well justified considering a comprehensive in vivo degradation study of PGS discs reported by Wang et al. Similar to many other studies on novel polyurethane elastomers, this study was also performed in comparison to PLGA as control. PGS lost weight steadily while the mass loss of the PLGA implant was first negligible (1% over 14 days) and then spiked to 61% within next 7 days. Another important finding of this study revealed a significant advantage of PGS: PGS degraded via surface erosion in contrast to PLGA that degraded via bulk degradation causing cracks about 20 µm wide within 2 weeks post-implantation. Another advantage of PGS is the preservation of implant geometry and retention of mechanical strength. This is an important feature of PGS, as one of the key functions of degradable polymeric biomaterials is to provide mechanical support during tissue regeneration. In comparison to the PLGA implants that have lost their physical strength after 7 days, the modulus of PGS decreased by less than 50% of the initially determined value. Similar to PGS, other sebacate elastomers also show desirable degradation patterns as the mass loss upon in vitro degradation reaches almost 80% after 4 weeks.

Apart from glycerol, difunctional SA can also react with other multifunctional alcohols in polyesterification reactions. Recent work of Bruggeman et al. presented the development of biodegradable polymers (poly[polyol-co-(sebacic acid)]s) based on polyesterification of SA with numerous multifunctional alcohols. The synthesized materials exhibited optimal material properties for cellular growth and high-level control was possible over physicochemical and degradation characteristics by simple alteration of five monomers (including SA) chosen for the polyesterification reaction.

The production of PGS has been extended further by the introduction of acrylate moieties that make the novel acrylate-functionalised PGS (PGSA) polymer photocurable with a wide range of mechanical properties. This material was tested in vitro with primary human foreskin fibroblasts, and results from cell attachment and proliferation assays indicated promising potential for skin tissue engineering. Cells attached and proliferated on PGSA films exhibiting healthy morphology in both initial stages of cellular growth (24 h) and after an extended period of time (12 days). Although this work, together with that on other photocurable polymer systems proposed for tissue engineering, has shown promising results, there are concerns in terms of cost-effectiveness and complexity of such fabrication processes, rendering them unsuitable for use in commercial clinical applications. Considering the simplicity of the synthesis process and the reactivity of both SA and CA with multifunctional alcohols, there are still a number of possibilities for combining biocompatible and non-toxic reactants in order to develop highly controllable biomaterials for future biomedical applications.

POLYDIOL CITRATE ELASTOMERS

CA is a metabolic product of the body via the Kreb cycle or tricarboxylic acid cycle and is, therefore, considered to be non-toxic. In aerobic organisms, the citric acid cycle is a part of the metabolic pathway involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water to generate a form of usable energy. Similar to SA, CA reacts with multifunctional alcohols generating elastomeric polymers with a high degree of crosslinking via tertiary –COOH groups (Fig. 1). Of particular interest is the POC material with the chemical structure shown in Fig. 1. Due to the optimal biocompatibility and moderate immunological response to the material, scaffolds fabricated from the POC elastic polyester are promising to be effective for regeneration of various types of tissues. In terms of the immunological response to the material, Yang et al. have investigated POC films implanted into Sprague–Dawley rats together with PLGA controls. No chronic inflammatory response was detected with minimal fibrous capsule formation measured to be 45 µm after 60 days. The fibrous capsule formed on the...
POC implants was thinner than the one observed on PLGA, which is in line with the results obtained for PGS.18,31

Owing to its elastic properties similar to those of PGS, the POC elastomer has found an application in blood vessel engineering.31–33 POC-based scaffolds fabricated using the solvent-casting/particulate-leaching technique are reported to be compatible with in vitro human aortic smooth muscle cells (HASMCs) and human aortic endothelial smooth muscle cells (HAEMCs).31 In vitro assessment of the POC elastomer designed for small-diameter blood vessel tissue engineering was reported by Yang et al.33 A new process has been developed for the fabrication of biphasic tubular scaffolds out of the POC elastomer designed for small-diameter blood vessel tissue engineering.33 The key reason behind this particular scaffold design is that the two phases of the scaffold should ideally provide the barrier function of the basic membrane for endothelial cell function (inner non-porous phase) and artificial elastic laminas for separation between cell layers (outer porous phase).33 In vitro results with HASMCs and HAEMCs seeded on tubular porous scaffolds and nonporous films expressed differentiated phenotype indicating potential to function as a tissue unit.33 This finding could be of significant importance for cardiovascular surgery considering the difficulties in the engineering of small-diameter blood vessels.53

A similar biphasic system for small blood vessel tissue engineering was produced from PGS lumen (inner non-porous phase) and POC porous outer layer.53 This scaffold was tested in vitro for blood and plasma compatibility in comparison to polytetrafluoroethylene (PTFE) vascular grafts that were used as controls.53 Overall, the measured platelet adhesion, inflammatory potential and clotting indicated good haemocompatibility and clotting indicated good haemocompatibility and biocompatibility of biphasic tubular scaffolds out of the POC elastomer.31

Due to its reported biocompatibility and controllable degradation rates (a few months to a year), POC has potential for bone fixation applications.34 Another important advantage is that the POC–HA composite can be fabricated with controllable mechanical properties. In contrast to pure POC that is a soft and resilient elastic material (tensile Young’s modulus of ca 2 MPa),31,32 the POC–HA composite expressed controllable tensile modulus in the range 20–300 MPa corresponding to the HA concentration in the composite (40–65 wt%).34 An in vivo investigation showed well-integrated POC–HA implant with surrounding cartilage and bone morphology around the implant similar to that of normal bone.34

A general hypothesis adopted in the development of tissue engineering biomaterials is that control over the physicochemical properties of a material would ultimately lead to adequate control over its biological response.35 Regarding biocompatible polymer elastomers (such as PGS and POC), the simple polyesterrification process could provide the ability to closely control material properties such as hydration, elasticity and hydrolytic degradation. In addition to the POC elastomer that is produced in a polycondensation reaction between CA and OD, the CA monomer can react with a number of different diols thus producing a family of novel biodegradable materials referred to as polydiol citrates (PDCs).36 Yang et al. have synthesised PDC materials by reacting CA with diols in the range of C6 to C12.36 The main rationale for this idea is that the availability of various diols provides the flexibility to tune material characteristics such as mechanical integrity and biodegradability.36 Similar to the SA-based polymer system,44 processing and control over PDC polymers are achieved by utilising more than three monomer units which add to the complexity of such systems.36,44 Another important aspect of potential combination between sebacate and citrate polyesters is that both SA and CA can react simultaneously with one particular multifunctional alcohol, where the tertiary –COOH groups of CA are less likely to be esterified.45

While the class of polymer elastomers discussed above has shown extensive promise for biomedical applications, the following section presents some key findings regarding the novel tunable polyester system, i.e. p(OCS), that has recently been developed as a copolyester with randomly distributed CA/SA segments.47–49

**RANDOM COPOLYMER P(OCS)**

The novel class of p(OCS) polymers developed by us was synthesised by polymerisation between CA and OD monomers.47

A range of CA/SA initial molar ratios with fixed OD concentration were investigated. The nomenclature for this polymer system is given in Table 2, together with some material properties.47 The versatility of the p(OCS) materials was also established through various processing techniques such as complex film design, porous scaffold fabrication and thin-film coating.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Initial molar ratio OD : CA : SA</th>
<th>Young’s modulus (MPa)</th>
<th>Hydration (%)</th>
<th>Degradation (4 weeks, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p(O(C1S0))</td>
<td>1 : 1.0</td>
<td>0.19</td>
<td>15</td>
<td>70</td>
</tr>
<tr>
<td>p(O(C0.75S0.25))</td>
<td>1 : 0.75 : 0.25</td>
<td>0.20</td>
<td>9</td>
<td>64</td>
</tr>
<tr>
<td>p(O(C0.5S0.5))</td>
<td>1 : 0.5 : 0.5</td>
<td>1.10</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

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The first stage of the synthesis involved the preparation of pre-polymers by melting mixtures of monomers at high temperatures (140–160 °C, 1 h). The pre-polymers were then dissolved in dioxane to obtain their solutions, which were subsequently used for making films and thin-film coatings by solvent casting and spin coating, respectively. The p(OCS) films for physicochemical analyses were produced by solvent casting of pre-polymer solutions of the various p(OCS) compositions (Table 2). Pre-polymers were cured in Teflon casts at moderate temperature (80 °C) for 7 days and the films thus obtained were further processed by compression moulding (150 °C and 15 MPa) to reduce their thickness to the sub-millimetre range. All the films (Fig. 2(A)) were produced under the same reaction conditions. Pre-polymer solutions of p(OCS) were also used to produce thin-film coatings by spin coating on silicon wafers and microscope glass slides. The spin-coated substrates were cured at 80 °C for 7 days for subsequent surface analysis and in vitro biocompatibility tests.

p(OCS) elastomers were also successfully processed in the form of porous scaffolds (Figs 2(B)–(D)) using a modified solvent-casting particulate-leaching technique. In brief, a solution of the synthesised p(OCS) pre-polymer was mixed with salt and the polymer/salt composite slurry was poured into a Teflon cast and left in an oven for further polyesterification (curing). During this stage, both the solvent and water (product of the polyesterification reaction) were allowed to evaporate from the system. During the curing process, short polymer chains (pre-polymers) reacted via their terminal –COOH and –OH functionalities causing further polyesterification. The final result was the formation of longer p(OCS) chains crosslinked around the salt particles. The particles were later washed out by extensive soaking in water.

**Polymer structure**

The relationship between the structure and properties of the synthesised p(OCS) polyester elastomers was revealed through polymer characterisation. Of particular interest for the identification of polymer chemistry is matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-ToF-MS) analysis that was used to investigate the p(OCS) pre-polymer structure, and it was concluded that the distribution of CA/SA segments can be closely controlled by simple variation of the initial CA/SA molar ratio of the monomers. Here we describe the MALDI-ToF-MS analysis of the p(OCS) elastomers in some detail.

Over the last decade, MALDI-ToF-MS has been successfully used for investigating both biological and synthetic polymers. In particular, end-group analysis and distribution of units along polymer chains can be accurately determined using MALDI-ToF-MS. The MALDI-ToF-MS spectra of p(OCS) polymers were obtained after a number of experimental trials during the optimisation process. The optimisation process involved several experiments where samples were prepared by conventional methods in which all the components were first mixed together (polymer, matrix and ionising agent) and subsequently the mixture was deposited on the MALDI-ToF-MS plate. All such experimental trials failed to result in any useful MALDI-ToF-MS spectra, regardless of sample/matrix/ionising agent/solvent variation in the sample mixtures under described experimental conditions. After additional attempts, a layer-by-layer sampling method provided good-quality spectra.

Figure 3(A) shows a magnified MALDI-ToF-MS spectrum of the p(O_1C_0.75S_0.25) polyester. The first peak at m/z = 957 g mol⁻¹ shows the structure composed of two CA, one SA and three OD units as shown in Fig. 3(B). In the case of p(O_1C_0.75S_0.25) polyester, the molecular structure could be described as a random copolymer built out of six blocks (units). In such compositions, the OD represents the repeating unit between either of the acid units (CA or SA). Both the acid units can take random positions within the p(OCS) polyester chains detected in our MALDI-ToF-MS experiments. The spectrum in Fig. 3(A) shows peaks with intervals of Δm/z = 10. This is most likely a result
of the formation of different structures depicted in Fig. 3(B). Put simply, the molecular weights of CA and SA are 192 and 202 g mol\(^{-1}\), respectively, and when those two units form polymer structures, they give rise to peaks with values of \(m/z = 957\) and 967 g mol\(^{-1}\) (Fig. 3(B)). Further esterification of the terminal acid groups, i.e. the addition of OD group to both the copolymer species from Fig. 3(B), is detected by two peaks in the second series at \(m/z = 1085\) and 1096 g mol\(^{-1}\), where CA and SA units can have exchanged positions formed in polyesterification reaction. Similar to the MALDI-ToF-MS spectra of the other p(OCS) structures investigated by us, the p(O\(_{0.75}S_0.25\)) polymer composition shows the formation of \(-\text{COO–Na}\) complexes marked with double-headed arrows in Fig. 3(A). For example, the sequence following the peak at \(m/z = 1085\) can be described...
as 1085 + 22n where n = 0, 1, 2, 3. This molecule has two free –COOH groups and has two terminal OD units with free –OH groups. Both the functionalities interact with Na⁺ as described above.

Polyesterification reaction between CA and SA and the chosen non-toxic OD monomer, where the number of CA segments in the polyester chain is closely controlled, yields citrate/sebacate polyesters with controlled number of –COOH groups. These functionalities contribute to the crosslinking density of p(OCS), resulting in the variation of glass transition temperatures (T_g) determined by DSC. All the synthesised p(OCS) polyesters show T_g below room temperature, a characteristic feature that determines their elastomer-like behaviour. In the case of p(OCS), T_g progressively increases with increasing initial concentrations of CA in the reaction mixture: −37, −18 and −7 °C for p(O1C0S0S0), p(O1C0S2S0) and p(O1C1S0), respectively. Polymer chains formed from the pre-polymers with higher number of CA segments are expected to have strong intermolecular interactions, resulting in reduced molecular segmental dynamics and increasing T_g. When the temperature was increased above T_g, the molecular motion in the p(O1C0S0S0) sample allowed further molecular organisation and exhibited cold crystallisation followed by DSC. This particular sample displayed crystalline melting at T_m ≈ 28 °C, which was also confirmed by visual observation. When held at room temperature within the range 20–25 °C, the p(O1C0S0S0) sample appeared as a cloudy, non-transparent white rubber-like material (Fig. 4), which retained its elastic integrity similar to the other samples (Fig. 2A). However, on heating above 28 °C, the sample instantly became transparent, indicating a transition from partial crystallinity to an amorphous state (Fig. 4). The semi-crystalline morphology of p(O1C0S0S0) is most likely a result of the formation of longer aliphatic segments generated by polyesterification between OD and SA. In order to confirm that no unreacted monomers were present in the polymers, DSC experiments were also performed in one heating cycle (25–170 °C). No new peaks due to the presence of unreacted monomers were detected.

The highest value of Young’s modulus was recorded for the p(O1C0S0S0) sample (Table 2). This particular structure also exhibits the highest elongation during tensile stretching (ca 230%). Due to the semi-crystalline nature of this particular polymer composition, p(O1C0S0S0) represents boundary conditions between elastomeric and crystalline polymers. An increase in Young’s modulus is characteristic of an increase in intermolecular hydrogen bonding within the examined material. In the case of p(O1C0S0S0) polyester, the high level of intermolecular bonding is most likely a result of close packing of molecules within the crystalline segments.

Swelling of these elastomers in water reveals how equilibrium percentage swelling decreases with decreasing concentration of CA (Table 2). This particular result suggests that in the case of p(OCS) polyester elastomers, hydration is dependent on the number of hydrophilic functionalities such as carboxyl and hydroxyl groups present. The swelling experiment also demonstrates that the samples with longer hydrophobic aliphatic chains have less percentage swelling in water. The sample with composition p(O1C0S0S0) shows the lowest hydration due to strong presence of aliphatic hydrophobic segments generated from polyesterification between OD and SA. In the present case, replacing CA with SA as a building block in copolyester formation led to decreased swelling in water, i.e. by simply varying the CA/SA ratio in the pre-polymer composition. The results obtained from hydrolytic degradation experiments corresponded to the swelling data recorded for p(OCS) polymers (Table 2). As expected, the p(O1C0S0S0) elastomer displayed the slowest degradation rate due to the existence of longer hydrophobic chains that did not allow molecules of water to penetrate into the structure thus causing the cleavage of ester bonds. The variation of pendant –COOH functionality on p(OCS) polymer chains does not just contribute to the crosslinking density of such a polymer system (resulting in tunable hydration and hydrolytic degradation rates) but also presents polymeric surfaces with controllable surface concentration of –COOH groups.

Detailed surface analysis of the p(OCS) elastomers (prepared by spin coating) is presented below in order to show the correlation between surface properties and controllable p(OCS) polymer composition.

**Surface properties of p(OCS) elastomers**

Characterisation of polymeric biomaterial surfaces and the nature and density of inherent functional groups in them are crucial for improving their function in tissue engineering applications. It has been shown that free surface carboxyl (–COOH) groups can interact with different cell types in either inhibitory or encouraging fashion. Li et al. reported that –COOH groups have an inhibitory effect on cell attachment and differentiation of neural cells. On the other hand, keratinocytes and osteoblast-like cells exhibit
strong affinity towards substrates with –COOH surface groups as a result of improved hydrophilicity.\textsuperscript{64,65} Recently, gradients of carboxylic acid groups have been used to control neuronal cell differentiation and behaviour\textsuperscript{64} and embryonic stem cell pluripotency.\textsuperscript{62}

Another important feature of such functionalised surfaces is that the –COOH groups can be used for covalent attachment of ECM proteins, growth factors and protein sequences that provide tissue guidance during the regeneration process.\textsuperscript{61} Numerous chemical processes have been developed to achieve covalent attachment of biomolecules (such as proteins or peptide sequences) onto biologically inert polymer surfaces.\textsuperscript{61} At this stage, it is important to mention that the optimisation of functional group surface density is more important than its maximisation. For example, too many functional groups on a biomaterial surface can lead to overcrowding of proteins attached, thus causing reduced biological activity due to steric hindrance or changed conformation.\textsuperscript{61}

In some instances, polymeric materials used for biomedical applications (including tissue engineering scaffold fabrication) may require surface modification or functionalisation to improve the surface properties of the materials. This includes modification of surface chemistry\textsuperscript{46} and topography,\textsuperscript{25,61} and immobilisation of biomolecules.\textsuperscript{61} These procedures are often necessary when precise control over cell functions, such as attachment/adhesion, proliferation and differentiation is required on a bioactive surface. However, any post-synthesis modification procedure adds to the complex and cost of the material, thereby reducing its potential for use in clinical applications. Considering the chemical structure of POC in particular (Fig. 1), the appearance of –COOH groups is expected on the polymer surface due to the tertiary –COOH group originating from the CA segments.\textsuperscript{36} Although this expectation has been highlighted in some of the relevant publications,\textsuperscript{31,36,41} no attempt was made to quantify this functional group. Through toluidine blue assay, recently we have quantified the available surface functional groups on p(OCS).\textsuperscript{48}

Figure 5 shows the micro-wrinkled surface morphology of p(OCS) elastomer (recorded using SEM) typical for elastomeric materials. The surface chemistry of p(OCS) was studied using X-ray photoelectron spectroscopy (XPS) and the curve-fitting of the p(OCS) C 1s peak was also performed (Fig. 5(C)). The results summarised in Table 3 confirm that the surface chemistry of the p(OCS) elastomers can be closely controlled. The unique feature of p(OCS) is that the surface density of the –COOH groups can be easily tuned on demand by simply varying the initial acid concentration ratio (CA/SA) in the pre-polymer during synthesis.\textsuperscript{48} The water-in-air contact angle was measured on p(OCS) surfaces and data correspond well with the results from XPS analysis (Table 3). Put simply, p(O1C1S0) composition with the highest concentration of –COOH surface groups was found to be the most hydrophilic out of the three investigated p(OCS) compositions. Such an approach in polymer synthesis may prove advantageous for fine-tuning biomolecule loading and release by varying the swelling and degradation rates, modifying the mechanical properties for elastic vascular tissue or varying the surface concentration of –COOH groups to control cell function.\textsuperscript{47,48} All of the p(OCS) polyesters tested were non-toxic and could support cellular growth,\textsuperscript{49} as summarised in the following section.

**In vitro cell compatibility of tunable p(OCS) elastomers**

Experimental trials were first performed on p(OCS) polymer discs with varying polyester compositions (10 mm diameter and ca 1 mm thickness) seeded with 3T3 fibroblast cells. The discs required extensive washing prior to cell culture in order to remove any soluble diol/citrate/sebacate oligomers that had been detected in the swelling-in-water experiment (2–5% of soluble material).\textsuperscript{47} This soluble material proved toxic for the cells due to the decreased pH in the culture medium. Further optimisation experiments were

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**Table 3.** Relative concentrations of the various components curve-fitted to C 1s XPS spectra and water-in-air contact angle recorded for the various p(OCS) samples\textsuperscript{a}

<table>
<thead>
<tr>
<th>Polymer</th>
<th>–C–O–C–</th>
<th>–COOH</th>
<th>–C–O–C==O</th>
<th>Contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p(O1C1S0)</td>
<td>20.7</td>
<td>17.5</td>
<td>31 (±2)</td>
<td></td>
</tr>
<tr>
<td>p(O1C0.75S0.25)</td>
<td>13.7</td>
<td>14.8</td>
<td>41 (±3)</td>
<td></td>
</tr>
<tr>
<td>p(O1C0.3S0.7)</td>
<td>10.3</td>
<td>13.5</td>
<td>64 (±3)</td>
<td></td>
</tr>
</tbody>
</table>

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**Figure 5.** Surface properties of the p(O1C0.75S0.25) polyester elastomer, SEM images of (A) the cross-section and (B) the surface. (C) XPS profile of the curve-fitted C 1s photoelectron. (Reprinted with permission from Djordjevic \textendash; Polym Int 2010, VSP, Leiden.)
Poly[octanediol-co-(citric acid)-co-(sebacic acid)] elastomers

Figure 6. Phase contrast images of 3T3 fibroblast cells grown on (A) TCPS control and (B) p(O1C0.75S0.25) polymer disc surface (images taken after 2 days in culture).

Figure 7. Phase contrast micrographs of MG63 cells on p(OCS) films after 4 h in culture: (A) p(O1C1S0); (B) p(O1C0.75S0.25); (C) p(O1C0.5S0.5); (D) TCPS control. Elongated, flattened and circular cell morphologies are marked with solid arrows, dashed arrows and circles respectively. The scale bar applies to all micrographs. (Reprinted with permission from Djordjevic et al.49 Copyright 2010, VSP, Leiden).

Conducted and it was concluded that the p(OCS) samples required washing for at least four days prior to cell seeding, two days in phosphate-buffered saline and two days in serum-free cell growth medium. After this washing step, the cells attached and grew on the p(OCS) polymers for longer periods of time, as shown in Fig. 6.

The application of p(OCS) as a scaffolding material for bone tissue engineering was examined using human MG63 osteoblast-like cells (Fig. 7). A similar relatively high density of MG63 cells had attached and formed normal spindle-shaped osteoblast morphology on all three of the test materials after 4 h in culture, with only a few cells remaining circular (marked with black circles in Fig. 7). Furthermore, two types of spindle-shaped cell morphologies have been detected: cells that express elongated narrow structures; and cells that appear to be flattened (marked with solid and dashed arrows in Fig. 7). MG63 cellular vitality was examined using the alamar blue assay. No statistical difference was observed in the number of viable cells on the p(OCS) films and the tissue culture polystyrene (TCPS) control after one day of cell culture. Interestingly, following four days of cell culture, the relative cellular vitality was found to be statistically greater on the p(O1C0.75S0.5) and p(O1C0.5S0.5) samples compared to the p(O1C1S0) sample, which is known to be a biocompatible material for supporting the growth of osteoblast cells. The measured cell vitality on the p(O1C0.5S0.5) polymer composition was similar to that on the TCPS control and the highest number of viable cells was measured on the p(O1C0.75S0.25) sample compared to the other samples tested in this experiment. There are multiple chemical and physical factors that could have contributed to the difference in cell growth as observed on the p(OCS) elastomers. For instance, it is possible that the p(OCS) materials which contain SA (p(O1C0.75S0.25) and p(O1C0.5S0.5)) and possess higher Young's modulus, elongation and tensile strength, reveal a...
surface resembling closer the ECM surrounding the native cellular environment.  

CONCLUSIONS
The novel citrate/sebacate copolypesters are elastomeric in nature and their material characteristics, such as strength, elasticity, hydration and hydrolytic degradation, are strongly influenced by their structure. It is evident that design of such polymer systems can be elegantly controlled by means of chemical synthesis. The results obtained from the morphological and surface chemistry studies of p(OCS) reveal that the bulk material properties and the inherent functional group density are dependent on the initial concentrations of the constituent monomers. The p(OCS) polymers in their coating form, tested in vitro, support the growth and proliferation of human-derived bone-forming cells (MG63). Cell culture tests showed optimal performance of all three p(OCS) polymer compositions displaying cellular attachment and healthy phenotype. The cell culture results clearly showed that simple alteration of the p(OCS) composition influenced cellular growth on the polymer surface, a feature advantageous for synthetic tissue engineering materials. These in vitro results provide substantial evidence that the p(OCS) polyesters expand the repertoire of available biodegradable polymers suitable for bone tissue engineering. For future work on p(OCS), scaffold fabrication using the various chemical compositions should be pursued aimed at engineering of large-volume tissues. Multiphase scaffolds can be developed with p(OCS) layers of different compositions. Knowing the one p(OCS) structure supports cell proliferation better than another, layers can be designed to initiate cell migration and proliferation within three-dimensional scaffold structures. Protein activation of (p(OCS)) surfaces should also be considered. The presence of varying carboxyl group concentrations could lead to effective attachment of specific proteins or protein sequences. A desirable outcome would be the creation of protein gradients that would result in precise control over cellular migration and proliferation within the scaffold volume.

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REFERENCES
Poly(octanediol-co-(citric acid)-co-(sebacic acid)) elastomers
