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Dynamic mechanical properties of hydroxyapatite-reinforced and porous starch-based degradable biomaterials

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It has been shown that blends of starch with a poly(ethylene-vinyl-alcohol) copolymer, EVOH, designated as SEVA-C, present an interesting combination of mechanical, degradation and biocompatible properties, specially when filled with hydroxyapatite (HA). Consequently, they may find a range of applications in the biomaterials field. This work evaluated the influence of HA fillers and of blowing agents (used to produce porous architectures) over the viscoelastic properties of SEVA-C polymers, as seen by dynamic mechanical analysis (DMA), in order to speculate on their performances when withstanding cyclic loading in the body. The composite materials presented a promising performance under dynamic mechanical solicitation conditions. Two relaxations were found being attributed to the starch and EVOH phases. The EVOH relaxation process may be very useful *in vivo* improving the implants performance under cyclic loading. DMA results also showed that it is possible to produce SEVA-C compact surface/porous core architectures with a mechanical performance similar to that of SEVA-C dense materials. This may allow for the use of these materials as bone replacements or scaffolds that must withstand loads when implanted.

1. Introduction

There is a need for the development of new biodegradable materials to be used in orthopaedics and as scaffolding for hard tissue engineering [1–5]. An ideal material for this type of application should associate a dynamic mechanical performance matching that of the tissue to be replaced (regenerated), an adequate degradation behavior (function of the tissue healing kinetics) and a biocompatible performance [1, 2, 5–7].

Is has been shown that blends of starch with poly(ethylene-vinyl-alcohol) copolymer, EVOH, designated as SEVA-C, can associate a biodegradable behavior with an interesting mechanical performance [7–10]. These materials have been extensively studied in the last few years, by our research group. It has been shown [9, 11] that the mechanical properties can be enhanced by incorporating bone-like inorganic fillers, such as hydroxyapatite (HA) or bioactive glasses. The production of HA-reinforced composites allows simultaneously for the development of bioactive [11] and biocompatible composites [12, 13]. In fact, according to the tests realized so far, the biomaterials based on SEVA-C blends do not induce any cytotoxic response from a range of cell lines [12, 13] and give raise to an unusual, for biodegradable polymers, biocompatible response when implanted in Dutch milk goats [12, 13]. These type of composites are expected to have potential use in filling bone defects, consolidating bone fractures and as bone plates or screws.

On the other hand, the introduction of blowing agents during injection molding can lead to the production of (porous core/compact surface layer) architectures more similar to bone morphology [14, 15]. This approach is being tested in order to develop melt processing routes for the production of scaffolding for the tissue engineering of bone [14, 15] or bone replacement materials [7–11]. The developed porous architectures can be used as tissue engineering scaffolds, drug delivery carriers or as cancellous bone replacements.

Dynamic mechanical analysis (DMA) is a very suitable technique for investigating the viscoelastic properties of polymeric materials in a wide range of temperatures and frequencies [16, 17]. It may be sensitive to structural environment at different space scales, from molecular level to the macrostructure of the specimen (for example, in composite materials) [18].

Furthermore, DMA measures the dynamic response of a particular polymeric system, being specially adequate to evaluate the performance of biomaterials when working under the cyclic solicitations generated by the human body physiological movements. Although human tissues are viscoelastic materials, DMA studies are not usual on biomaterials development investigations.

The main objective of this work is to study the influence of HA fillers and of blowing agents over the dynamical mechanical properties of starch-based biomaterials and to speculate on their possible performances when withstanding dynamic loading as implants in the human body.

2. Materials and methods

The studied polymer system was a blend of corn starch with poly(ethylene-vinyl alcohol) copolymer 60/40 mol/mol (SEVA-C), supplied by Novamont, Novara, Italy, under the trade name Mater Bi 1128RR. A general characterization of the properties of this blend may be found in reference [8]. SEVA-C blends were either: (i) reinforced with different amounts of HA (Plasma Biotal, Tideswell, UK) or (ii) foamed with a blowing agent (Hostalen P9937 system Hoescht, Germany) during injection molding. The HA was previously sintered at 1200 °C for 12 h, and then crushed in a ball-mill. After this procedure the HA powders presented a granulometric distribution with 90% of the particles below 7 μm (laser granulometry data). The experimental plan included the preparation of compounds with 0, 10, 20 and 30% (wt) of HA. The compounding procedure was based on twin screw extrusion (TSE) in a co-rotating Leitztritz equipment. The foamed materials were produced using either 10% or 20% by weight of blowing agent (BA).

Both type of materials were injection molded into adequate test specimens (dumb-bell specimens with $2 \times 4\,\mathrm{mm}^2$ cross-section) in a Klockner-Ferromatik Desma FM20. The processing conditions were optimized to ensure the maximum possible stiffness. More detailed information on the compounding and injection molding of the composite materials and on the production of foamed SEVA-C materials may be found elsewhere [7, 9–11, 14–15]. Also, an extensive characterization of the properties, *in vitro* and *in vivo* behavior of this type of blend, either unfilled or filled with HA or bioactive glasses, was reported before [7–15].

The dynamic–mechanical characterization was carried out under a three-point bending loading scheme using a Perkin-Elmer DMA7e apparatus with controlled cooling accessory. Temperature was scanned from -20 to $+120$ °C at $4\,°\mathrm{C\,min}^{-1}$ at a frequency of 1 Hz. The samples were placed over a 15 mm bending platform and a 5-mm knife-edge probe tip provided the mechanical excitation (a static stress of 1.20 MPa and a dynamic stress of 1.00 MPa). During temperature scanning the equipment is able to monitor the complex modulus, E^* and the loss factor, tan δ:

$$E^* = E' + \mathrm{i}E'' \qquad \tan\delta = E''/E' \qquad (1)$$

where $\mathrm{i} = (-1)^{1/2}$, E' and E'' are the storage and the loss moduli and δ is the phase angle between the dynamic strain and stress.

Quasistatic tensile tests were also performed in a controlled environment (23 °C and 55% RH) using an Instron 4505 universal mechanical test machine fitted with a resistive extensometer (gauge length, 10 mm). The molded specimens were previously conditioned at 23 °C and 55% HR for 2 weeks. The stiffness of several samples was characterized by means of the secant modulus at 1% strain $(E_{1\%})$. The idea was to compare, in a qualitative way, the data obtained under a flexure dynamic solicitation with that of standard quasistatic tensile data.

3. Results and discussion

Virgin SEVA-C presents two main relaxational processes in the temperature region -20 to 120 °C as seen by DMA, at frequencies around 1 Hz. Those processes are related with the onset of specific molecular motions, leading to modification upon the material's viscoelastic features in the temperature axis. For instance, the storage modulus, E', usually presents sudden decreases which alter the mechanical performances of the material. For the case of SEVA-C this diminution is found to be quite smooth. In Fig. 1 it can be observed that between 0 and 90 °C, E' decays *c.* 10 times. This is of much less significance than what is observed around the glass transition region, in most standard purpose synthetic thermoplastics.

The two above referred to relaxations are much more obvious in the E'' or tan δ plots. The relaxation at 30 °C is clearly detected in the E'' response. The attribution of this process is not straightforward due to the very complex structure of the material. The relatively high activation energy ($\sim 62\,\mathrm{kcal\,mol}^{-1}$) [19] indicates that this involves cooperative molecular diffusion and could be assigned to the glass transition of the copolymeric phase. The relaxation at 90 °C exhibits an even higher activation energy ($\sim 105\,\mathrm{kcal\,mol}^{-1}$) and has been attributed to molecular mobility within the starch fraction [19].

The lower temperature relaxation may be of high importance upon the potential biomedical applications of these materials. In fact, this dissipation phenomenon,

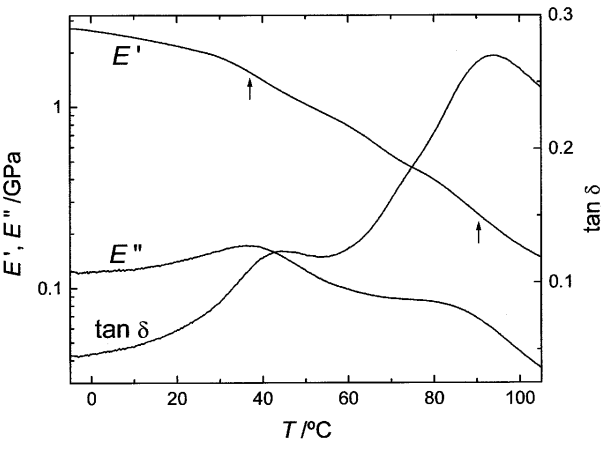

Figure 1 DMA spectrum of virgin SEVA-C at 1 Hz. The arrows indicate the temperature location of the two main relaxations.

which occurs near physiological temperature, will eventually enhance the implant performance when under the cyclic stresses that are characteristic of patient movements during daily activities. In this context it is worth remembering that the loss modulus is related to the mechanical energy which is dissipated during the periodic mechanical load, whereas E' measures the stored energy [16]

$$\varepsilon_{\mathrm{d}} = \pi E''\sigma_0^2 \qquad \varepsilon_{\mathrm{st}} = E'\sigma_0^2/4 \tag{2}$$

where ε_{d} is the energy dissipated during each cycle and per volume unit, $\varepsilon_{\mathrm{st}}$ is corresponding stored energy and σ_0 is the stress amplitude.

The dynamic mechanical response of SEVA-C changes with the introduction of HA. The results are shown in Figs 2 and 3. As expected, one observes a systematic increase of E' for higher filler contents. The results at 23 °C are also plotted in Fig. 4. In this figure the E' data obtained on DMA experiments is compared with the $E_{1\%}$ results from quasistatic tensile tests. The increase of E' with the introduction of 10% HA is around 10%, being 19% for 20% HA composites and 23% for 30% HA composites. On tensile static tests the effects of HA are much more pronounced. In fact, for the same HA concentration, the stiffness increases are 2.3 to three times greater for tensile experiments than for DMA flexural tests. However, as the two types of tests are based on quite distinct solicitation schemes, and consequent stress fields, one should not compare the results as absolute values. Nevertheless, it can be considered that any difference on the evolution of the two data sets can be qualitatively compared. Therefore, comparison between the available data may only conclude that the effect of the filler is less pronounced when the material is tested on a flexural dynamic mode than in standard tensile tests. This is a clear indication that the investigation of mechanical properties of novel biomaterials must include a variety of tests that should allow for the prediction of their performances under real conditions, that is in complex stress situations.

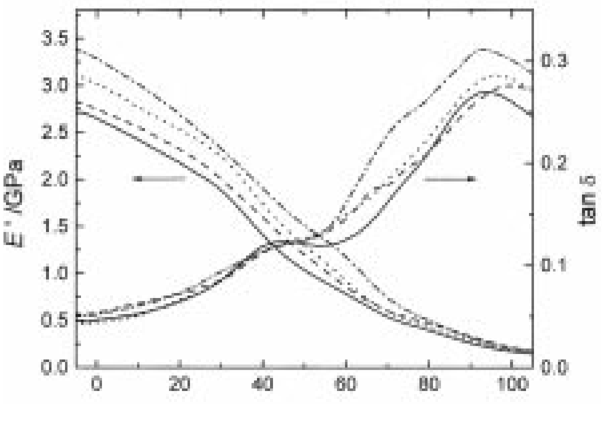

Figure 2 Storage modulus and loss factor of the SEVA-C/HA composites for different amounts of HA (wt%): (——) 0; (- - - -) 10; (· · ·) 20; (· - · - ·) 30.

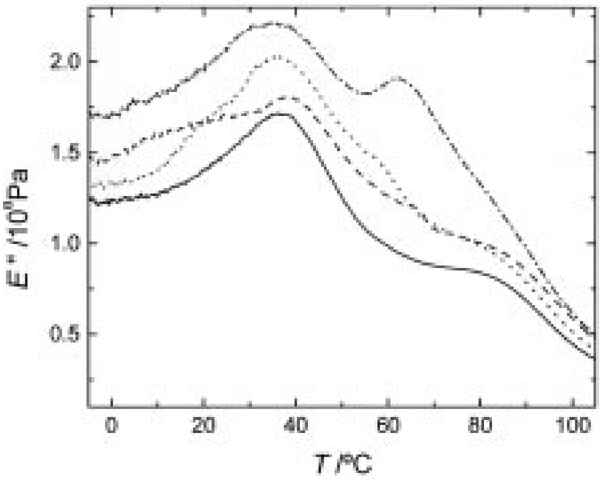

Figure 3 Loss modulus of the the SEVA-C/HA composites for different amounts of HA (wt%): (——) 0; (- - - -) 10; (· · ·) 20; (· - · - ·) 30.

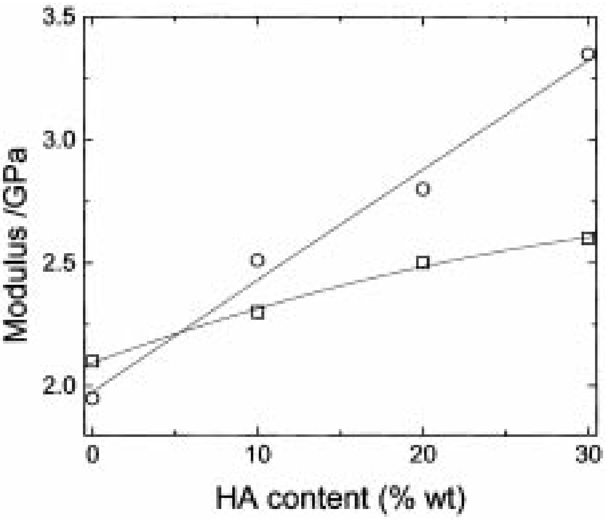

Figure 4 Stiffness of SEVA-C/HA composites at 23 °C. Squares: flexural storage modulus, E', at 1 Hz. Circles: tensile modulus at 1% strain, $E_{1\%}$.

These materials are complex interpenetrating polymeric networks filled with ceramic particles compounded by an intermeshing technique [10]. As a consequence, they are heterogeneous and even more dependent on the morphological development during processing than the mainly commonly used semicrystalline polymers. Although the system seems quite homogeneous (see fracture surface on Fig. 5a), it has been shown in previous works [9, 10] that the mechanical properties of this type of composite are deeply dependent on the processing conditions. So, speculation on the evolution of the plots of Fig. 4 should be avoided. Also, one should remember how different the results obtained in dynamical flexural tests can be, and in a standard tensile experiment for most thermoplastics. Nevertheless, it can be said that DMA is also a useful technique to estimate the stiffness increase caused by the HA addition.

In both E'' and tan δ plot it can be observed that the temperature of maximum intensity of the 30 °C peaks does not undergo any change. This is an indication that the inclusion of HA particles does not alter significantly the dynamics of the molecular mechanisms involved. Only a clear increase of the intensity of the E'' peak with increasing HA content may be observed. This is a consequence of the increase of the storage modulus and

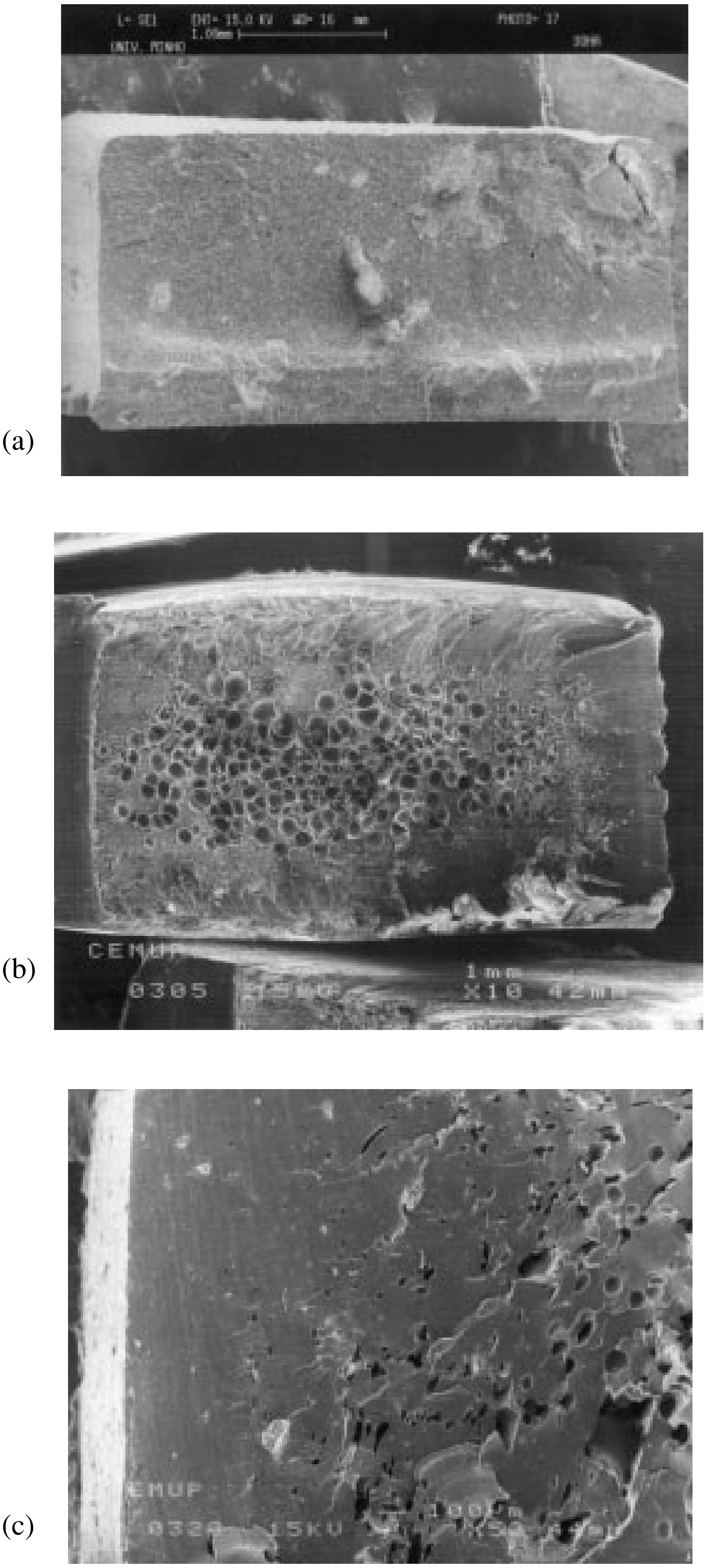

Figure 5 (a) Tensile fracture surface of a SEVA-C + 30% HA composite; (b) tensile fracture surface of a foamed SEVA-C with 20% BA; (c) magnification of (b) showing the very compact sample surface and the beginning of the foamed porous core.

the invariance of tan δ. From Equation 2 one concludes that the ability for the material to absorb and dissipate cyclic mechanical energy by means of the molecular motions assigned to the relaxation at 30 °C increases with higher HA concentrations.

The inclusion of HA also affects the relaxation at 90 °C. The tan δ peak broadens with increasing HA content. The results suggest that a new relaxation process emerges at 60 °C. This process is more visible for 30% HA and in the E'' plot of Fig. 3. This phenomenon may be caused by the scission or other degradation processes in the molecular fraction involved in the relaxation at 90 °C.

It is expected that the increase of viscosity and shear stresses during the processing of the material with increasing HA augments viscous dissipation within the molecular medium, promoting some thermomechanical degradation. In fact this type of blend has proved to be very sensitive to processing induced degradation [9–10, 12]. The process at 60 °C may be then assigned to the motions of the degraded fraction of the material involved in the process at 90 °C. It is interesting to note that the process at 60 °C also appeared for SEVA-C with greater moisture contents [19], thus promoting hydrolysis and macromolecular scission. This may have implications on the *in vivo* degradation of SEVA-C/HA composites, and even on its non-cytotoxic behavior, as shown before that thermally degraded samples will release faster a greater amount of low molecular chains to the physiological media [12–13, 20]. This will create osmolarity changes and a pH drop that may lead to cell morphological changes, and eventually cell death. *In vivo* an inflammatory response can eventually be induced by the fast release of these low-molecular weight chains. Consequently, the processing conditions of HA-filled composites should be optimized to avoid, as much as possible, the herein reported thermomechanical degradation.

Figs 6 and 7 present the flexural dynamic mechanical response of porous SEVA-C, with different blowing agent contents. In the studied temperature range no significant systematic changes are observed in the storage modulus spectra. Therefore, the increase of internal porosity does not compulsorily lead to a much less stiff material, when subjected to flexural stresses. These results are consistent with those previously [14–15] obtained on tensile static measurements, where there was no significant decrease of the modulus for foamed materials up to a 20% BA concentration. This may be explained by an additional holding pressure effect coming from a bulk pressure field due to the decomposition and consequent expansion of the BA, that tends to create a more dense and orientated skin that compensates for the detrimental effect of the internal porosity. In Fig. 5b and c it may be observed that the porous structure appears mainly in the sample's core. On these figures the very compact and oriented surface layer of the foamed SEVA-C materials is very apparent. This type of morphology may be useful on the development of bone analogue replacement materials and highly porous scaffolds that might be used on tissue engineering applications that must withstand load-bearing situations.

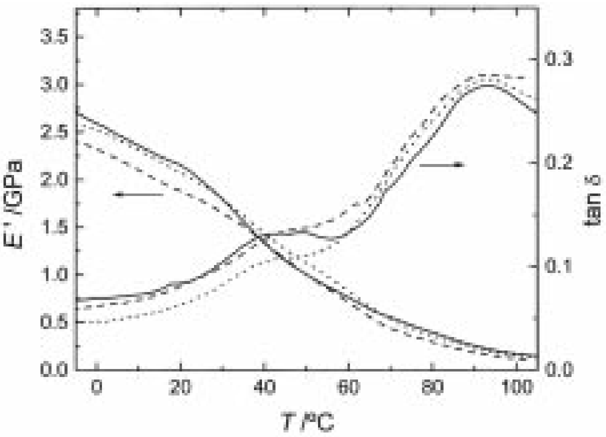

Figure 6 Storage modulus and loss factor of SEVA-C foams for different amounts of blowing agent (wt %): (——) 0; (- - - -) 10; (· · ·) 20.

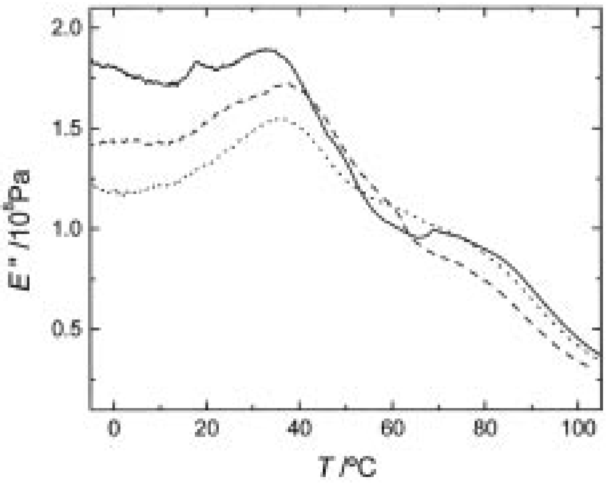

Figure 7 Loss modulus of SEVA-C foams for different amounts of blowing agent (wt %): (——) 0; (- - - -) 10; (· · ·) 20.

The tan δ and E'' plots show that the positions of the peak corresponding to the two relaxation processes do not present any changes in the temperature axis. The only remarkable modification is the strength of the relaxation at 30 °C which appears to decrease with increasing BA content. This may be explained by the smaller volume of SEVA-C which is able to dissipate the mechanical energy by means of the corresponding molecular motions assigned to that relaxation. It must be pointed out that the lower ability for the relaxation at 30 °C to dissipate energy does not mean that porous SEVA-C has less energy absorbing capabilities at those temperature regions. In fact it is well known that cellular materials are intrinsically good energy absorbers.

4. Conclusions

The SEVA-C/HA composite materials present a promising performance under dynamic–mechanical solicitation conditions. That may allow for their use as implant materials. The observed relaxation at ∼ 30 °C may be very useful *in vivo,* improving the implants performance when under cyclic loading. DMA results confirm that SEVA-C matrices with a porous core exhibit flexural mechanical properties that are very similar to those of SEVA-C compact moldings. Consequently these materials may find uses as tissue engineering scaffolds aimed at use on load bearing sites and/or critical size defects.

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