Laser stereolithography and supercritical fluid processing for custom-designed implant fabrication

V. K. POPOV*, A. V. EVSEEV
Institute on Laser and Information Technologies, Russian Academy of Sciences, Pionerskaya 2, Troitsk/Shatura, Moscow Region 142092, Russia
E-mail: popov@laser.ru

A. L. IVANOV, V. V. ROGINSKI, A. I. VOLOZHIN
Moscow Centre of Paediatric Crania-Maxillofacial Surgery, Moscow, Russia

S. M. HOWDLE
School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, UK

This paper describes the laser photopolymerization of a liquid mixture of polyfunctional acrylic monomers, photoinitiator and hydroxyapatite (HA). Pure polymeric and composite materials of specific shape and structure were fabricated by laser stereolithography based on images derived from three-dimensional (3D) computer modeling. The polymeric objects then were treated with supercritical carbon dioxide to remove potentially toxic residues (monomers, low molecular weight oligomers, etc.) and to provide interconnective microporosity. Finally, samples were implanted into white rats (diastolic epiphysis of femoral bone) to study living tissue response and processes of osteointegration and osteoinduction. It was shown that incorporation of HA into the composite implant structure encouraged periosteal as well as endosteal osteogenesis and improved their osteointegrative characteristics in particular. Supercritical carbon dioxide treatment significantly enhanced the biocompatibility of the materials, increasing the area of direct contact of the implant surface with regenerated bone tissue.

1. Introduction

Design and synthesis of advanced materials for hard tissue engineering and replacement is one of the main objectives in biomaterial research worldwide. The clinical success of implants or bone substitutes requires the simultaneous achievement of a stable interface with living tissue and a match of the shape and mechanical behavior of the implant with the bony tissue to be replaced.

Fabrication of medical implants for clinical application is a good example of a custom-designed production. Every single implant requires an individual shape to be directly implanted into a specific patient at a particular site. Laser stereolithography is a key technology for rapid fabrication of material copies of three-dimensional (3D) computer images [1–3] and this technique shows great promise for individual implant fabrication. The stereolithography process is based upon photopolymerization of photocurable resins (PCR) initiated by laser radiation [4]. This technology and commercially available equipment allow the fabrication of polymer prototype of real objects with dimensions up to $25 \times 25 \times 25\ cm^3$. This size range permits fabrication of a copy of almost any fragment of the human skeleton.

Computerized data obtained from X-ray, NMR or ultrasonic tomography can be utilized to make implants [5–7]. Advanced software permits editing of the computer images. Thus one can re-introduce missing structures, or remove undesirable bone fragments in order to optimize the desired shape for a new implant. The global ''Internet'' network allows surgeons to send such computer files from remote clinics or hospitals to the laser stereolithography machine to start custom-designed implant fabrication without delay. Such processing has been used successfully to aid surgeons in visualizing operating procedures by creating solid 3D models of the implants and/or site of operation [8–11].

However, there are important limitations in this technology that have prevented direct implant. Generally, only a few special acrylic and epoxy-based monomers have the appropriate photoabsorption characteristics to allow effective stereolithography photopolymerization to take place. These polymers were never optimized for biomedical applications *in vivo*. In

*Author to whom all correspondence should be addressed.

addition, a major drawback of the acrylic polymer objects produced is that they are substantially contaminated by residual unreacted monomers, low molecular weight oligomers, initiators and binding agents. These residues can dramatically affect the biological response of tissues and cell cultures to the fabricated polymeric materials. Thus, their application in clinics can often be accompanied by various post-operational inflammatory and dystrophic processes [12]. All of these factors mitigate against the use of stereolithography for development of synthetic scaffolds for tissue engineering and guided bone regeneration and hence explain why this technology has not yet been developed further to produce real medical implants.

In this paper we present the results of our study of a new material and new methods for manufacturing custom-designed 3D mineral–polymer composite scaffolds for guided bone regeneration and cranio-maxillofacial implants. Our work has focused on polyfunctional methacrylic oligomers and mixtures of these with bioactive hydroxyapatite (HA). Our approach is based on the unique combination of two techniques; laser stereolithography for rapid prototyping of 3D computer images [13] and supercritical fluid processing to effectively remove toxic residues from the polymer composites. In addition, the same supercritical fluid processing provides a novel way of introducing the interconnected microporosity required for bone-forming cell attachment, growth, proliferation and differentiation [14].

2. Materials and methods

Acrylic polymers have been widely used in medicine over the last 50 years as bone cements and as components for implant materials. However, because of their intrinsic toxicity and relatively poor mechanical properties they are still far away from the ideal material for implant fabrication. Recent research in polymer chemistry has yielded new, less toxic materials with improved mechanical properties (e.g. stiffness). In particular, polyfunctional methacrylic oligomers have shown success and are characterized by high reactivity and good cross-linking abilities [11, 12]. It is the properties of the initial oligomers and parameters of the polymerized cross-linked structure, which determine the mechanical and biochemical characteristics of the final product.

Olygocarbonatedimethacrylate (OCM-2™) (synthesized by Institute of Chemical Physics, Moscow, Russia) was the main component of the PCR used in our experiments. OCM-2™ can be effectively polymerized by a radical mechanism and its characteristics are given in Table I. 2,2-Dimethoxy-2-phenylacetophenone (Irgacure 671™) was utilized as a photo-initiator for radical polymerisation. To prevent spontaneous polymerization, (bis-(5-methyl-3-tert-butyl-2-oxyphenyl)-methane has been used as an inhibitor. Monodisperse (ca. 1 μm) hydroxyapatite powder ($Ca_{10}(PO_4)_6(OH)_2$) – Hydroxypol™ was synthesized and delivered by ''Polystom Ltd.'' (Moscow, Russia). It has been used as bioactive and reinforcing mineral filler for PCR.

TABLE I OKM-2™ (PCR) characteristics

Molecular mass, M_w	419
Functionality	1.96
Refractive index, n_D	1.466
Density, g cm^{-3}	1.209
Viscosity, cSt	260
Inhibitor concentration, wt %	0.02

The LS-250 apparatus was used for laser stereolithography. This is commercially available equipment from Institute on Laser and Information Technologies (Shatura, Russia) and is shown schematically in Fig. 1. In order to build up a 3D object, a computer image of the object A was first sectioned into thin (~200 μm) slices B, which were reproduced in sequence on the surface of liquid PCR in the bath (4) by the focused HeCd laser (1) beam. As the laser beam draws the contour of the image, a very thin layer (ca. 0.1 mm) of solid polymer is built up. As a result of polymerization the solid polymer cross-section of the object is formed. A precision ''elevator'' (3) then moves the platform (5) of the bath down to allow the next layer to be drawn on top. The intensity of laser beam and velocity of its motion on PCR surface actually determine the rate of the individual layer formation. The process continues until hundreds of layers have been laid down to produce a 3D object with an accuracy of 0.1–0.2 mm. The shapes produced can be far more complicated than could ever be prepared by conventional, for example, molding techniques. As an illustration of power of the technique, Fig. 2 shows a picture of a skull biomodel made using X-ray tomography data [13].

In the conventional version, laser stereolithography leads to production of pure polymeric objects. Our aim was to develop a method for incorporating solid particles of other bioactive materials into the photopolymerization process so that the final product is a polymer-based composite material. Introduction of hydroxyapatite into 3D polymeric materials should substantially improve both composite mechanical properties and its interaction with existing and regenerating bone tissue in the body. Moreover, these properties should be tuned simply by varying the amount of HA loaded into composite. The key question is how such particles can be introduced into the stereolithography procedure. Our approach has been

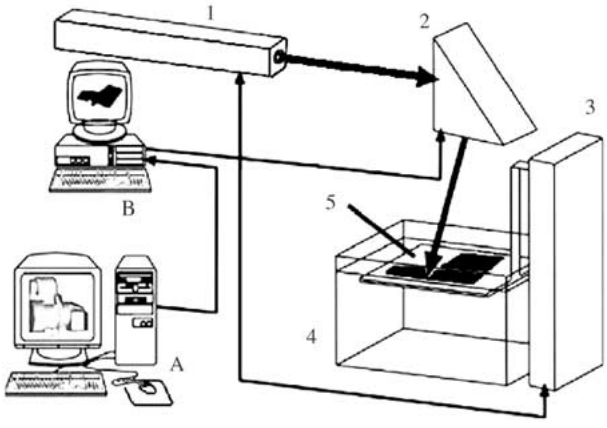

Figure 1 Laser stereolithography process. Computer image A of the object is sectioned into thin (~ 200 mm) slices B, 1 – HeCd laser, 2 – beam delivery system, 3 – precision ''elevator'', 4 – bath with liquid PCR, 5 – movable platform.

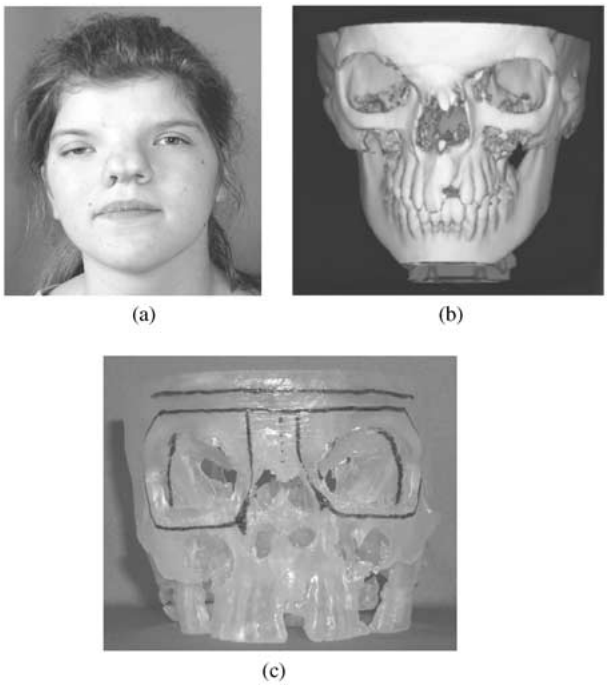

Figure 2 Laser stereolithography for biomodeling. A – 15 year old girl with front-nasal gap and hypertelorism; B – 3D computer tomogram; C – Biomodel with the lines for osteotomy.

to make up homogeneous mixture of HA particles with liquid monomer solution by simple stirring. This method was used to fabricate composite 3D objects. In our experiments, two types of implant were produced for *in vivo* study: cylinders (with diameter 1.5 mm and 3 mm length) and plates ($30 \times 10 \times 1.5\,mm^3$). For control samples, similar objects were fabricated from pure PCR without the inclusion of HA.

After the stereolithography step, all samples were treated with supercritical carbon dioxide (sc-CO_2) to enhance their biocompatibility by removal of toxic residues and to introduce microporosity [14]. All samples were placed into a custom-built stainless steel autoclave (maximum pressure and temperature – 300 bar and 200 °C) connected to the constant sc-CO_2 flow system interfaced with computer controlled flash valve and backpressure regulator. Alteration of processing parameters (pressure, temperature, time duration, rate of depressurization) allows manipulation of the sample morphology and specific porosity.

The initial and sc-CO_2 treated polymer and composite samples were implanted into the white rat (line ''Vistar'', 250–270 g by weight) into the diastolic epiphysis of femoral bone. Rats were then sacrificed at 2, 4 and 8 weeks by overdose of hexenalum to evaluate the kinetics of cellular response and tissue reconstruction process. The epiphysizes were separated and fixed in 10% neutral formalin. The sections were coloured by hematoxylin-eosine. Scanning electron microscopy, histological and histochemical analyses have been applied to investigate the morphology and structure of the tissue/implant interface.

3. Results and discussion

The laser stereolithography process could indeed be performed on the suspension of HA particles within the

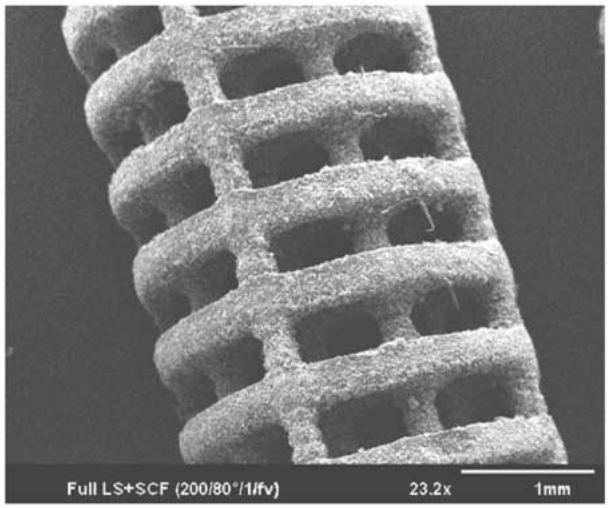

Figure 3 Composite HA(30 wt %)/OCM-2™ samples made by Laser Stereolithography.

liquid PCR. However, there is a limitation. The density of hydroxyapatite ($\rho_{HA} = 3.2\,g\,cm^{-3}$) is about three times higher than the density of acrylic PCR ($\rho_{PCR} = 1.2\,g\,cm^{-3}$). Over a period of time the suspended HA begins to settle out by sedimentation from the liquid monomer. Our experiments have shown that small objects that take less than 60 min to fabricate can be easily produced by this technique (see Fig. 3). However, if the object is larger, or more complex, the processing takes longer than 1 h and then agglomeration and sedimentation of the HA particles substantially affects the quality of the finished object, resulting in a non-uniform distribution of HA particles throughout the object and partial deposition of HA powder at the bottom of the liquid monomer bath. Moreover, introduction of HA into the liquid PCR significantly increased its viscosity and this was found to impede the formation of each new layer of liquid PCR with required thickness (0.2 mm) on the solid composite surface. Our experiments show that the limiting concentration of HA that can be used in our experiments is ca. 30 wt % of the mixture. Clearly, the size of the suspended HA particles will affect the rate of sedimentation, but this provides only limited control. We now propose a modification of the method designed to circumvent the sedimentation problem.

Previously, we have found that pre-treatment of HA powder with polyacrylic acid (PAA) or a polymer complex – polyacrylic acid/polyvinylpyrrolidone, leads to substantial increases in the interaction of the particles with a polymer host (PMMA). This results in a significant improvement of the bending and impact strength, as well as, microhardness of PMMA/HA composites [15].

We believe that the polymer coating is bound to the HA particles by interaction of the carboxyl group of PAA with the HA. Thus, the polymer coated HA now more efficiently interacts with the PMMA host through polymer–polymer interactions at the coated particle/host interface. In the present work we have applied the same principle to modify the HA particle surface prior to mixing with liquid PCR. The results demonstrate that not only the mechanical properties of the final composite

material are enhanced, but also a substantial (factor of 3) decrease is observed in the rate of HA particle sedimentation in the liquid bath. This is significant because in lengthens dramatically the period over which the stereolithography process can be performed successfully. In addition, the coating of the HA particles also has the effect of substantially reducing by ca. 70%, the viscosity of the suspension of HA particles in PCR. Thus, we have been able to increase the loading of HA in the suspension to as high as 40–45 wt % without adversely affecting the stereolithography process. This dramatically broadens the range of composites and mechanical properties that can be accessed. We are currently further exploring the precise effects of such chemical modification of HA on the sedimentation process, and the results will be reported elsewhere.

By using these modifications we have used stereolithography process to produce a range of novel craniomaxillofacial HA composite implants with specific shapes and with homogeneously distributed HA particles at up to 40 wt % throughout the composite. Most importantly, the decreased sedimentation rates allowed very complex shapes to be produced in a procedure carried out over a period more than 3 h (Fig. 4).

In addition to the novel stereolithography process, we have also developed post-processing with $scCO_2$. Controlled porosity of implants or scaffolds is one of the key factors required for successful clinical application, but such defined microporosity (less than 0.1 mm) cannot yet be achieved directly by laser stereolithography. However, the supercritical fluid technique provides a solution. $ScCO_2$ is an unusual solvent with the properties of both liquid and gas [16]. Like a liquid $scCO_2$ can plasticize polymers, but like a gas it penetrates much more effectively into polymeric and porous materials. The interaction of CO_2 with the polymer chains leads a lowering of the glass transition temperature (T_g) and plasticization. Under these conditions, it has been demonstrated that the $scCO_2$ pressure can be carefully released under controlled conditions to yield microcellular foamed polymeric materials such as poly(acrylates) or polystyrene [17, 18]. We have now demonstrated that the same level of control can be applied to the HA/polymer composites described in this study. Manipulation of the CO_2 density (temperature and pressure) and the rate of depressurization allow fine control of the size, distribution, and total pore volume, as well as their interconnectivity The pore structure obtained is extremely well defined with predominantly small (ca. 5–30 μm) pores (Fig. 5).

In our previous studies [19, 20], we have also demonstrated that the same $scCO_2$ processing step can completely remove any toxic residues by extraction from PMMA/HA composites and this can indeed dramatically improve the biocompatibility of the materials. Our present experiments demonstrate similar results. Without $scCO_2$ extraction implants made without HA (i.e. polymer only) provoke significant inflammation (in particular in the first few weeks after implantation) and are found to be separated from the surrounding tissues by an intervening fibrous capsule which varies in thickness

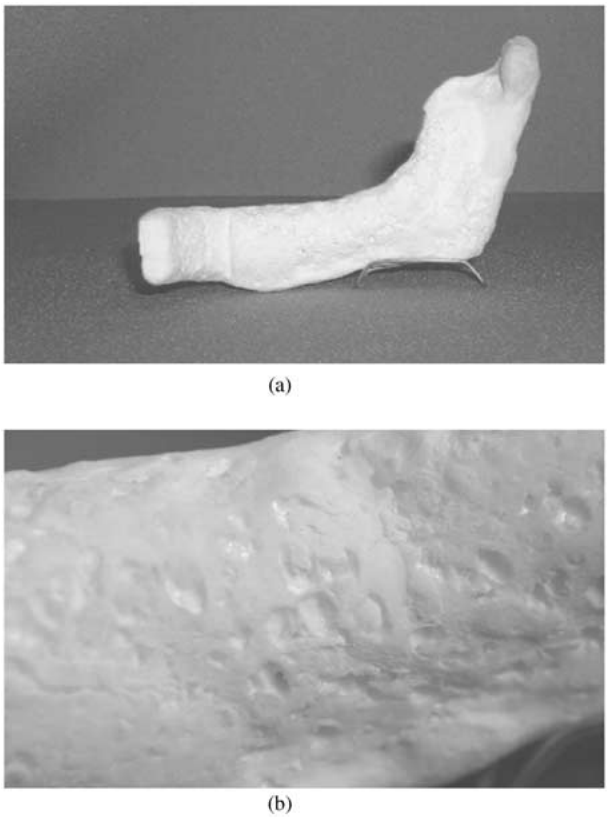

Figure 4 Composite implant for mandibular part reconstruction (A). B – close-up.

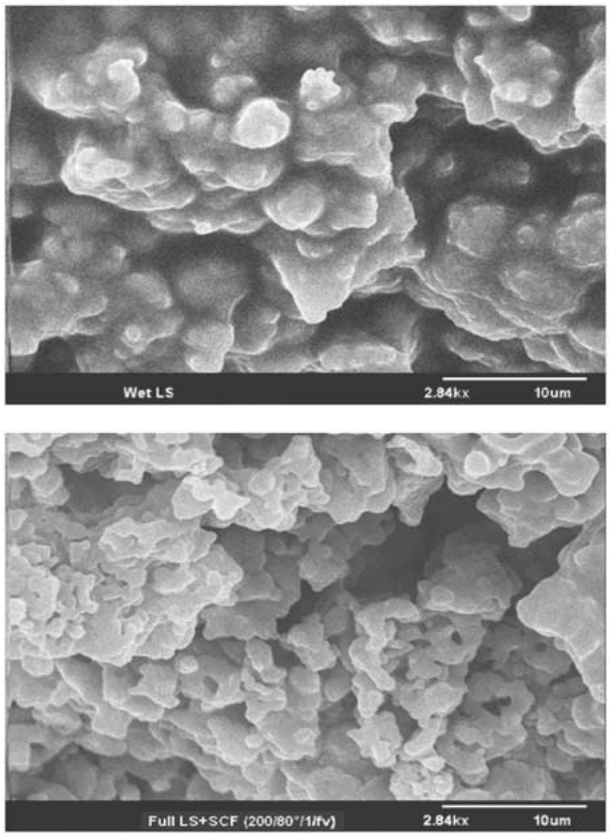

Figure 5 OCM-2℠/HA composite sample before (upper) and after (lower) sc-CO_2 treatment. The introduction of the microprous structure by $scCO_2$ can be clearly visualized. In addition, the process also removes residual toxic materials.

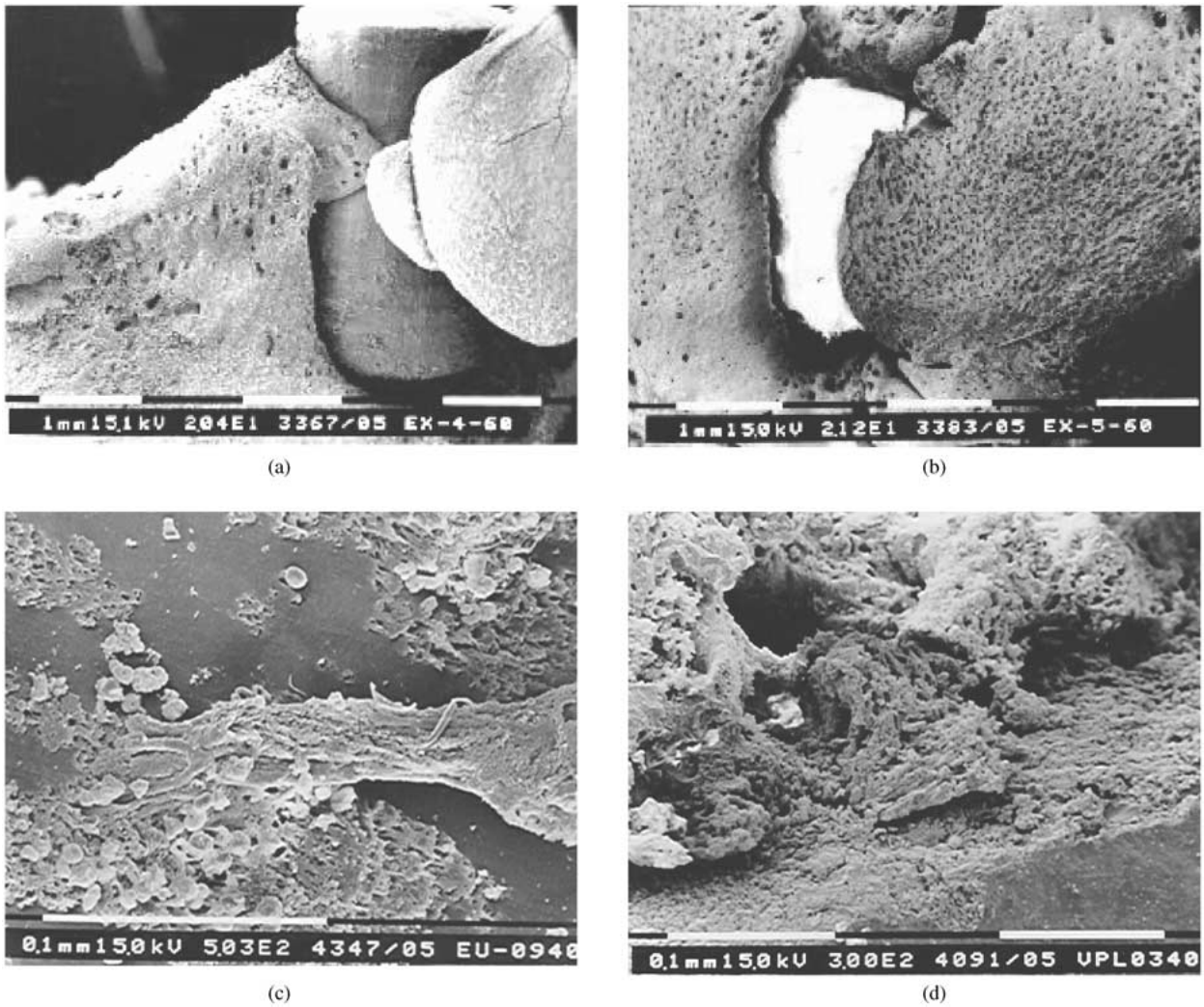

Figure 6 SEM images of pure polymer (a) and polymer/HA composite (b–d) interfaces with regenerated bone tissue. a–c before and d after sc-CO_2 treatment.

from sample to sample (Fig. 6(a)). Even after 8 weeks, no direct contact of new bone trabecula with the polymer surface could be observed. The stereolithographic composites of polymer and HA are little better and show only very limited areas of regenerated bone tissue in direct contact with the implant surface (Fig. 6(b)). In fact, the trabecula attached only to HA clusters in the composite structure (Fig. 6(c)). In some cases, extensive sites of mineralised cartilage between the implant surface and regenerated bone structures were observed.

By contrast, the use of $scCO_2$ extraction dramatically alters the picture. All of the extracted samples show negligible inflammatory infiltrates and dystrophy changes into surrounding and regenerated tissues. This is particularly true for the composites which contain HA. It was clear that for theses samples extensive periosteal and endosteal osteogenesis was present at the implant sites. After four weeks, the implant surfaces were covered with trabecula spongy structures and showed large areas of direct contact (Fig. 6(d)). After a period of 8 weeks following implantation these samples were surrounded by a dense cortical layer of regenerated bone which was strongly integrated into the body of the implant prepared by stereolithography.

4. Conclusions

Laser photopolymerization of polyfunctional methacrylic oligomers and their mixtures with bioactive hydroxyapatite has been studied. Pure polymeric and composite materials of specific shape and structure were fabricated by laser stereolithography based on 3D computer modeling. Toxic residuals (monomers, low molecular weight oligomers, etc.) have been extracted with supercritical carbon dioxide leading to very clean materials with specific microporosity. Finally, samples were implanted into the white rats. Diastolic epiphysis of femoral bone allowed study of the living tissue response and the processes of osteointegration and osteoinduction. It was shown that incorporation of HA into the composite structure encouraged periosteal as well as endosteal osteogenesis and particularly improved their osteointegrative characteristics. Supercritical carbon dioxide treatment significantly enhanced the biocompatibility of the materials increasing the accessible area of the implant surface in direct contact with regenerated bone tissue as well as removing any toxic residues within the composite structure. A key advantage of this technology is that any fragment of human skeleton can be reproduced directly based on computer tomography

data and then can be used in the operating theater without additional shaping.

Acknowledgments

The authors are grateful to Prof. A. P. Krasnov, O. V. Afonicheva and Dr O. Z. Topolnitski for their help in the development of HA modification, powder grafting and *in vivo* tests. The authors would like also to acknowledge the financial support of The Wellcome Trust for Collaborative Research Initiative Grant No 062760. S.M.H. is a Royal Society Wolfson Research Merit Award Holder.

References

1. P. F. JACOBS (ed), ''Rapid Prototyping & Manufacturing: Fundamentals of Stereolithography'', (SME, Dearborn, MI, 1992).
2. T. M. BARKER, W. J. EARWAKER and D. A. LISLE, *Australas Radiol*. **38** (1994) 106.
3. P. S. D'URSO, T. M. BARKER and W. J. EARWAKER, *J. Cranio-maxillofacial Surg*. **27** (1999) 30.
4. A. V. EVSEEV and M. A. MARKOV, *Quantum Electron.* **24** (1994) 454.
5. R. PETZOLD, H. F. ZEILHOFER and W. A. KALENDER, *Comput. Med. Imag. Graphics* **23** (1999) 277.
6. B. ISSA, P. GIBBS and R. HODGSKINSON, *Magn. Resonance Imag*. **16** (1998) 651.
7. C. M. LANGTON, M. A. WHITEHEAD, D. K. LANGTON and G. LANGLEY, *Med. Eng. Phys.* **19** (1997) 599.
8. A. I. VOLOZHIN and T. I. SASHKIN, ''Allergy and Other Forms of Intolerance in Dentistry'' (Nauka, Moscow, 1996).
9. P. S. D'URSO, R. L. ATKINSON and M. W. LANIGAN, *Br. J. Plastic Surg*. **51** (1998) 522.
10. S. M. HOWDLE, M. S. WATSON, M. J. WHITAKER, V. K. POPOV, M. C. DAVIES, F. S. MANDEL, J. D. WANG and K. M. SHAKESHEFF, *Chem. Commun*. (2001) 109.
11. A. A. BERLIN, G. V. KOROLEV, T. YA. KEPHELI and YU. M. SEVERGIN, ''Acrylic Oligomers and Material on Their Base'' (Russ.) (Khimiya, Moscow, 1983).
12. A. F. MASLYUK and V. A. HRANOVSKIY, ''Photochemistry of Polymerisable Oligomers'' (Russ.) (Naukova dumka, Kiev, 1989).
13. V. V. ROGINSKI, A. V. EVSEEV, E. V. KOTSUBA, V. K. POPOV, A. V. PASECHNIKOV, A. L. IVANOV and O. Z. TOPOL'NITSKI, *Paediatr. Dentist.* (Russ.) **1–2** (2000) 92.
14. A. I. VOLOZHIN, O. Z. TOPOL'NITSKI, V. K. POPOV, V. V. ROGINSKI, I. V. MATVEICHYUK, A. A. DOCTOROV, A. P. KRASNOV and T. T. BIRYUKBAEV, *News Dentist.* (Russ.) **3** (1999) 32.
15. A. P. KRASNOV, O. V. AFONICHEVA, V. K. POPOV and A. I. VOLOZHIN, *Int. J. Polym. Mater*. **53** (2004) in press.
16. M. A. MCHUGH and V. J. KRUKONIS, ''Supercritical Fluid Extraction; Principles and Practice'' (Butterwoth, Boston, 1994).
17. S. K. GOEL and E. J. BECKMAN, *Polym. Eng. Sci*. **34** (1994) 1137.
18. E. KUNG, A. J. LESSER and T. J. MCCARTHY, *Macromolecules* **31** (1998) 4160.
19. V. K. POPOV, E. N. ANTONOV, V. N. BAGRATASHVILI, YU. P. SUKHANOV, A. I. VOLOZHIN, A. B. SHECHTER, S. M. HOWDLE and D. JONES, *Med. Biol. Eng. Comput*. **35** (1997) 606.
20. V. K. POPOV, A. I. VOLOZHIN, A. B. SHEKHTER and M. CARROTT, *Dentistry* (Russ.) **77** (1998) 4.

Received 30 April
and accepted 10 September 2003