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BIOCOMPATIBILITY OF LASER-DEPOSITED HYDROXYAPATITE COATINGS ON TITANIUM AND POLYMER IMPLANT MATERIALS

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ABSTRACT

We have investigated the biocompatibility of calcium phosphate coatings deposited by pulsed laser ablation from hydroxyapatite (HA) targets onto polyethylene and Teflon substrates. It was found that the cell density, attachment, and morphology of primary rat calvaria osteoblasts were influenced by both the original polymer and by the nature of the apatite coatings. HA coatings on Teflon were found to have higher biocompatibility in terms of cell adhesion and spreading. *In vivo* studies of bone response to coatings deposited by KrF excimer and CO_2 lasers on commercial Ti6A14V alloy implants show that both deposition techniques suppress fibrous tissue formation and promote osteogenesis. © *1998 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(98)00704-7]

Keywords laser deposition; hydroxyapatite; polymers; biocompatibility; implantation; osteoblasts.

1 INTRODUCTION

Calcium phosphate plasma sprayed coatings on medical implant surfaces are known to accelerate bonding and new bone formation at the interface between the implant and the bone tissue.[1] Recently pulsed laser ablation deposition techniques have been developed to produce biocompatible coatings from hydroxyapatite targets.[2–4] Our previous analysis of laser-deposited hydroxyapatite (HA) coatings has mainly focused on *in vitro* studies of biocompatibility of the coatings on metallic substrates,[5,6] revealing that manipulation of the laser deposition conditions allows fine control of the biocompatibility of the surface coating.

One of the key advantages of the pulsed laser deposition technique over conventional plasma spray methods is that, during the deposition process, there is little heating, and the substrate temperature remains at or near room temperature. We have exploited this advantage to facilitate HA surface coating of polymeric materials.[7] We believe that this procedure will significantly widen the range of implant materials available for medical applications, and will complement existing methods for optimization of the mechanical and biochemical properties of polymeric implant materials.[8–13]

The aims of this work are twofold: to extend the technique of pulsed laser deposition by excimer laser to HA coatings of polymeric materials and to test the performance of HA coatings deposited using a TEA-CO_2 laser on conventional alloy materials.

2 METHODS

2.1 COATING DEPOSITION

Deposition of the HA coatings on metals and polymers was performed by laser ablation of HA targets in a stainless steel chamber with 2 Pa residual air pressure at room temperature. The deposition of HA coatings on Ti6A14V alloy substrates was used for a comparison study of coatings deposited with KrF-excimer laser pulses ($\lambda = 248$ nm, $t_{pulse} = 30$ ns) and TEA-CO_2 laser ($\lambda = 10.6\ \mu$m, $t_{pulse} = 100$ ns). The deposition parameters are given in Table 1. Our preliminary tests have shown that HA coatings deposited with a CO_2 laser are about one order of magnitude less stable in simulated body fluid (SBF) than those deposited with a KrF laser. To improve

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Table 1 Coating deposition parameters.

	Substrate	Laser	f (Hz)	Fluence (J/cm^2)	Thickness (μm)	Post-treatment
1	Teflon	KrF	2–5	3–9	1	
2	PE	KrF	2–5	3–9	1	
3	Ti6A14V	KrF	10	10	4	
4	Ti6A14V	KrF	10	10	4	550 °C, 1 h, 10^{-2} Pa
5	Ti6A14V	CO_2	10	10	4	550 °C, 1 h, 10^{-2} Pa

the stability of the coatings, the as deposited implants were annealed for 1 h at 550 °C and 10^{-2} Pa residual air pressure.

Note that we describe the coatings deposited from HA targets by pulsed laser ablation as HA coatings. However, the true composition of the laser-deposited coatings is a mixture of various calcium phosphates, of which calcium hydroxyapatite is only one. As shown in Ref. 4, the higher the laser fluence and/or gas pressure in a chamber, the closer the composition of the coating approximates the composition of the initial HA target.

2.2 DISSOLUTION TEST

The stability of laser-deposited HA coatings with respect to dissolution has been determined by Fourier transform infrared (FTIR) spectroscopy.[5] The relative film thickness (with respect to the initial sample) was determined from the integral intensity of the PO_4 absorption band at $\sim$1050 cm^{-1} during the dissolution process in free calcium SBF (0.2 g/l KCl, 0.2 g/l KH_2PO_4, 8 g/l NaCl, and 2.16 g/l $Na_2HPO_47H_2O$).

2.3 TOXICITY TEST

For the toxicity evaluation of the coatings, a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) photometric test was employed to assay mitochondrial enzyme activity. For this assay a 3T3 mouse fibroblast cell line was used; it was cultured in Dulbecco's minimum essential medium (DMEM) with 10% newborn calf serum, and maintained under normal cell culture conditions at 37 °C and 5% CO_2. The cells were cultured for 48 h on HA-coated and uncoated polymeric materials, including ''control'' tissue culture polystyrene, after which they were exposed to media containing 100 mg/ml MTT. As a negative control, some cells were cultured in varying concentrations of sodium docecyl sulphate (SDS). After a further 4 h of incubation the resultant colored product was dissolved in 0.4 ml/l 1 M HCl in dimethyl sulphoxide and the absorbance measured at a wavelength of 540 nm.

2.4 OSTEOBLAST GROWTH

A morphological assessment of the biocompatibility was carried out by scanning electron microscopy (SEM) using osteoblasts obtained from embryonic 20 day Wistar rat calvaria dissociated by collagenase/trypsin digestion.[14] Cell suspensions, containing 1.6×10^4 cell/ml in 20% fetal calf serum-supplemented DMEM, were seeded onto HA-coated Teflon and polyethylene (PE) samples (4 mm diam disks), and incubated for periods of up to 48 h. The samples were then fixed with 2.5% glutaraldehyde, postfixed with osmium tetroxide, and processed for SEM by dehydration and critical point drying.[15]

2.5 IMPLANTATION

The coatings deposited with KrF-excimer and TEA-CO_2 lasers (see Table 1) onto $2.5\times2\times1$ mm^3 Ti6A14V alloy implants that had been tested *in vivo*. The implants, both coated with HA and uncoated (control), were inserted into adult rat femurs (total number of rats: 36) for periods of 15, 30, and 60 days. The bone responses and new bone tissue formation were analyzed by SEM and by histological analysis.[16]

3 RESULTS

3.1 COATINGS ON POLYMERS

The dissolution kinetics of HA coatings on PE and Teflon substrates in SBF are shown on Figure 1. Each point shown on these kinetic curves is the result of averaging the relative film thickness over three identical samples in three different experiments. HA coatings deposited on PE and Teflon substrates under the same conditions were found to dissolve at very different rates. Indeed, coatings on Teflon substrates are much more stable than those on PE. The SEM micrographs of the coatings on Teflon substrates show a marked difference before and after erosion (Figure 2), namely, the apparent

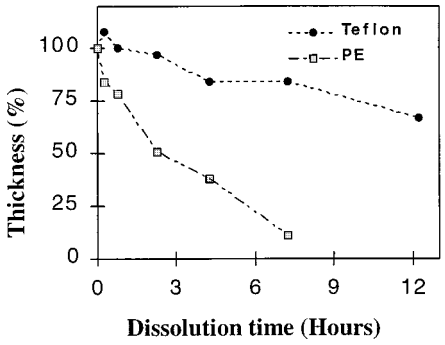
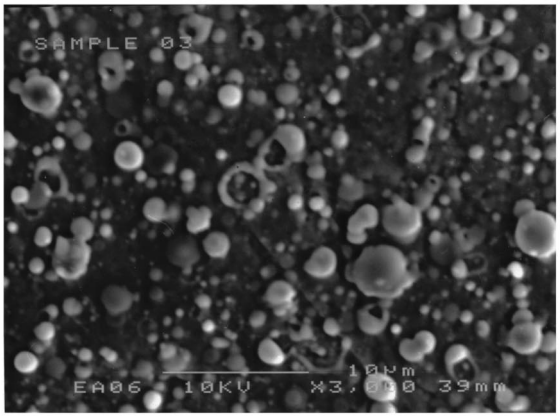


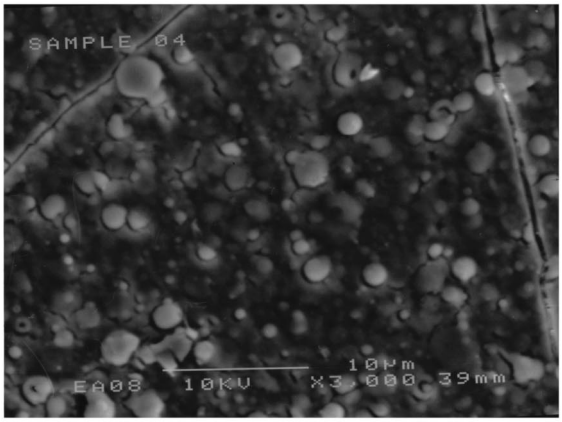
Fig. 1 Dissolution rates of HA coatings deposited on Teflon and PE substrates. Dissolution medium SBF; laser fluence 7.5 J/cm^2.

disappearance of a substantial portion of the macroparticles on the surface, presumably via dissolution.

Cytotoxicity data obtained by a MTT test on HA-coated and uncoated PE and Teflon substrates are shown in Figure 3. These results show that fibroblasts grown on HA-coated PE exhibit greater mitochondrial activity than those on the positive control, indicating that this surface is nontoxic.



(a)



(b)

Fig. 2 SEM micrographs of the HA coating on Teflon (a) before and (b) after soaking in SBF.

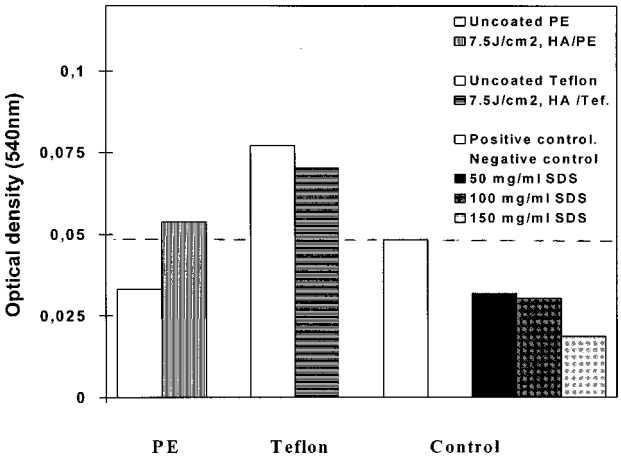


Fig. 3 MTT cytotoxicity test for polymers and HA coatings on PE and Teflon samples. The dashed line shows the neutral toxicity level.

Uncoated PE showed reduced mitochondrial activity, indicating some toxicity effects. Fibroblasts grown on HA-coated and uncoated Teflon exhibit greater mitochondrial activity than the positive control. There is, as expected, a reduction in the mitochondrial activity of cells grown in the presence of the negative toxic control containing 50–150 mg/ml SDS.

Morphological assessment of the biocompatibility of the surfaces was undertaken after a 48 h growth of primary rat calvaria osteoblasts. Figure 4 shows the mean osteoblast counts on the various substrates. SEM micrographs of osteoblasts grown on coated PE and Teflon (Figure 5) show that few, if any, of the attached osteoblasts could be detected on uncoated PE. In contrast, HA-coated PE demonstrates improved osteoblast growth over the uncoated samples. However, on closer observation, we see that many of these cells were rounded, indicating that they had not spread or attached well to the surfaces. Very different behavior was observed on the Teflon substrates, both coated and uncoated.

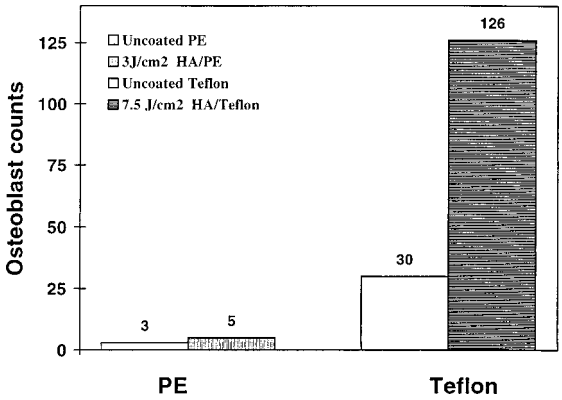
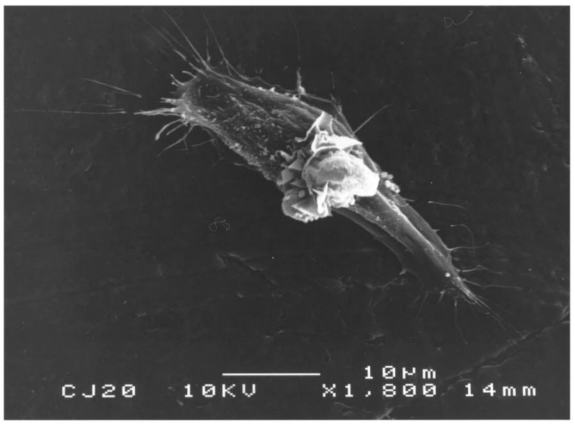
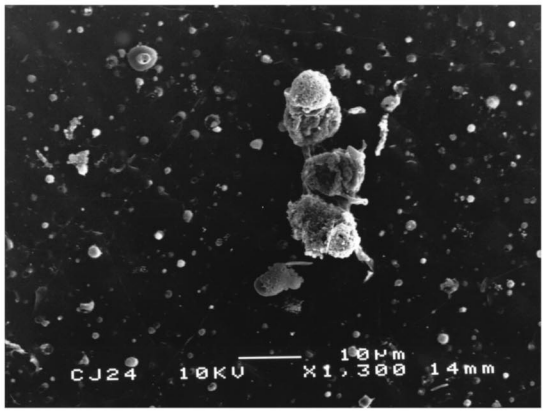


Fig. 4 Mean number of osteoblasts grown on uncoated and HA-coated Teflon and PE polymer surfaces. One quadrand of each of three samples was used for counting.

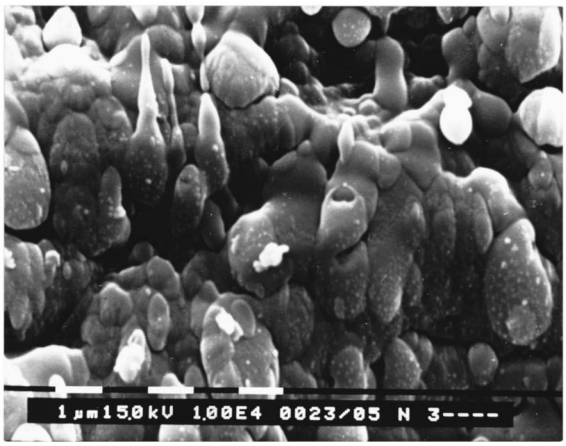


(a)

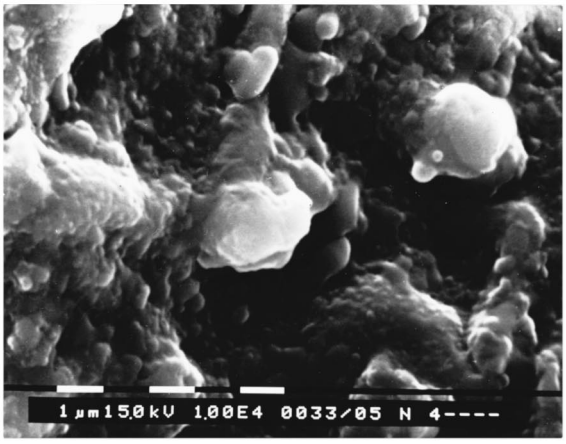


(b)

Fig. 5 SEM micrographs of osteoblasts grown on (a) HA-coated Teflon and (b) HA-coated PE.



(a)



(b)

Fig. 6 SEM micrographs of the annealed HA coatings deposited by (a) excimer laser and (b) CO_2 laser.

The cells on both samples showed high surface activity and growth. Addition of the HA coating to Teflon considerably increased the number of cells on the surface compared to the uncoated Teflon samples. Moreover, these osteoblasts showed clear signs of spreading and attachment to the HA/Teflon surface and numerous processes were observed between the cells.

3.2 COATINGS ON ALLOYS

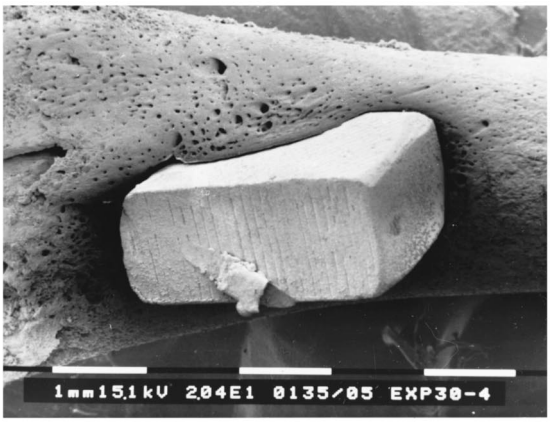
HA coatings deposited by KrF-excimer [Figure 6(a)] and CO_2 lasers [Figure 6(b)] on Ti6A14V alloy samples showed distinct morphological differences in their structures. The KrF laser-deposited coatings show more uniform macroparticle sizes whereas those deposited by the CO_2 laser are rougher, and with a much wider distribution. The macroparticle distributions before and after annealing are found to be very similar, but the annealing process does give rise to small crystallites on the surfaces of the existing macroparticles.

Finally we have investigated the *in vivo* behavior of these coatings through implantation studies. Our SEM studies (Figure 7) reveal new bone formation surrounding the site of implantation of the HA-coated alloy samples. Table 2 presents the results of a histological evaluation after 15, 30, and 60 days. No inflammation was observed around the sites of implantation for any of the groups of the samples, however, the uncoated samples did lead to significant fibrous tissue formation. In contrast, both the KrF laser and CO_2 laser coated samples exhibited very little fibrous tissue after 60 days and showed formation of new bone which was in direct contact with the implant surface.

4 DISCUSSION AND CONCLUSIONS

4.1 COATINGS ON POLYMERS

Our studies demonstrate that HA coatings deposited by KrF-excimer laser onto polymeric substrates lead to the formation of favorable surfaces for both fibroblasts and osteoblasts. The enhancement of cell bioactivity depends strongly on the stability of the coatings with respect to erosion. HA coatings deposited on Teflon surfaces appear to be more stable than those deposited on PE surfaces under identical conditions. The observed difference may well ac-



(a)

(b)

Fig. 7 New bone formation around implants after 60 days: (a) implant in a rat femur bone, (b) bone–implant interface view.

count for our observations of higher mitochondrial activity on the coated Teflon samples. This could be attributed to the fact that the HA coating on PE underwent extensive erosion during the culture period such that only about 10% of the initial coating thickness remained after 6 h of contact with SBF. These observations are also consistent with the apparent cytotoxicity of HA-coated PE in the MTT test (Figure 3) and with our previous data showing that delamination of HA from metal substrates leads to poor cell growth.[6]

The changes in bioactivity of HA coatings deposited onto different substrates are determined by physico-chemical properties of the laser deposited coatings, in particular, the composition, morphology, and adhesion. These properties are controlled both by properties of the substrate and by the laser deposition parameters. For each specific substrate material the parameters of laser deposition should be optimized. We believe that, in the case of Teflon, the parameters are close to optimal, but for the PE substrate further optimization is required.

4.2 COATINGS ON ALLOYS

The most significant advantage of HA coating on Ti6A14V alloy is the suppression of the fibrous tissue formation which is found around the uncoated metal implants *in vivo*. Thus, the laser deposited coating encourages early and long-lasting anchoring of implants in the bony cavity. No significant differences in the osteogenesis process were observed for samples coated by the KrF laser ($\lambda = 0.248\ \mu$m) or the CO_2 laser ($\lambda = 10.6\ \mu$m). In general, the osteointegration was slightly superior for the annealed rather than for the nonannealed samples. Coatings deposited by the CO_2 laser exhibit a markedly higher osteointegration rate than those from the KrF laser.

It should be noted that the use of TEA-CO_2 laser deposition of biocompatible coatings has not previously been reported. However, our results show that such lasers are highly effective, provided the deposition parameters are chosen properly. The high efficiency and relatively low cost of modern TEA-CO_2 lasers makes them a promising alternative for commercial development of laser ablation of HA coatings on medical and dental implants.

Acknowledgments

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Table 2 Histological evaluation of new bone growth.

	Deposition mode			
Implantation time	Excimer laser	Excimer laser + annealing	CO_2-laser+ annealing	Control samples
15 days	Thin fibrous layers			Hematoma
30 days	Thin fibrous layers; partially direct bone–implant contact			Fibrous tissue
60 days	New bone formation; direct bone–implant contact			Thin fibrous tissue

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