Cellulose Phosphates as Biomaterials. II. Surface Chemical Modification of Regenerated Cellulose Hydrogels

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ABSTRACT: Cellulose regenerated by the viscose process was previously investigated as an implantable material in orthopedic surgery. It was envisaged to take advantage not only of its good matching with mechanical properties of bone but also of its hydroexpansivity, therefore allowing a satisfactory fixation to hard tissue. Both the osteoconduction and the lack of osteoinduction of this material were demonstrated. Grafting of phosphate groups was then envisaged as the means to render cellulose more suitable for orthopedic applications by enhancing its bioactivity. In the present work, the previously optimized phosphorylation reaction was successfully adapted to the surface modification of regenerated cellulose. Modified materials were characterized by XPS, FTIR, and ^{31}P MAS NMR spectroscopic studies, and contact angle measurements, revealing the chemical bond between phosphate groups and cellulose, as well as the hydrophilic nature of phosphorylated materials, which increases with increasing phosphate contents. Water swelling and resistance to gamma sterilization were assessed as well, showing that phosphorylated materials swell considerably in water and were not affected when sterilization was carried out under a nitrogen atmosphere. The increase in surface roughness attributed to chemical modification was demonstrated through laser rugosimetry measurements. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 3354–3365, 2001

Key words: cellulose phosphates; biomaterials; phosphorylation; $H_3PO_4/P_2O_5/Et_3PO_4$/hexanol method; surface modification

INTRODUCTION

To fulfill the specific therapeutic needs of many existing diseases, new engineered biomaterials must be developed, specifically conceived for a required physiological response *in vivo.* A widely used concept concerns materials that are able to mimic living tissues.[1,2] Biomimetic materials are supposed to mimic some specific biological function. In the present case, it means a material capable of inducing the regeneration of bone tissue and of ensuring the whole, or at least part, of its physiological role. In orthopedics, it would be important to promote and/or accelerate the mineralization of decalcified tissues, as well as skel-

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etal implants, to ensure a satisfactory bonding at the interface between hard tissue and the biomaterial.

Polymers may be modified physically, chemically, or biochemically. Such modifications can have a significant influence on biological responses and on materials functionality.[3–5] Immobilization of biomolecules, as well as living cells and microorganisms, on solid or water-soluble supports, either in a temporary or a permanent way, is a promising approach for the preparation of biomimetic polymers. An important feature of the immobilization is often its attachment via a spacer group, which imparts greater steric freedom and thus greater specific activity.[3] Usually, in the case of biomaterials, the immobilization of biomolecules or cells to a polymeric support is preceded by the chemical modification of the support so as to provide reactive groups (e.g., —OH, —NH_2, —COOH).[3] Several modification techniques have been proposed for this purpose, including ionizing radiation graft copolymerization, plasma gas discharge, photochemical grafting, chemical modification, and chemical derivatization.[3–5] The development of surface modification techniques is making it possible to customize implant surfaces to specific requirements.

In a pioneer study, cellulose regenerated by a viscose process (CRV®) was successfully investigated as an implantable material in orthopedic surgery, as a sealing material for the femoral component in hip prostheses, in place of acrylic cement.[6–10] It was envisaged to take advantage not only of its good matching with mechanical properties of bone but also of its hydroexpansivity, therefore allowing a satisfactory fixation to hard tissue. The osteoconduction of this material was also demonstrated.[7–9] Nevertheless, a full bioactive character cannot be attributed to normally occurring cellulose because of its lack of osteoinduction.

To stimulate bone induction by CRV cellulose, its chemical modification via phosphorylation was envisaged. Once implanted, phosphorylated cellulose could promote the formation of calcium phosphates, thus having closer resemblance to bone functionality and ensuring a satisfactory bonding at the interface between hard tissue and the biomaterial. Furthermore, phosphate groups constitute adequate functionalities for specifically binding biologically active species,[11–17] and this can be used as an advantage for obtaining specifically customized active surfaces. In a previous work, the phosphorylation reaction was carried out by an original reaction route.[18,19] Reaction parameters were optimized using microcrystalline cellulose, and cellulose phosphates of high degrees of substitution were obtained.[18,19] Phosphorylated materials were characterized and were found not to be cytotoxic, in both cultured human osteoblasts and fibroblasts.[18–20]

In the present work, the technique optimized for obtaining highly phosphorylated gels, using microcrystalline cellulose, was applied to the surface modification of CRV cellulose, with the aim of enhancing its bioactivity. Modified materials were characterized by XPS, FTIR, and ^{31}P magic angle spinning (MAS) NMR spectroscopies, and contact angle measurements. The water swelling and the resistance of this material to gamma sterilization were also assessed, as well as changes in surface roughness resulting from chemical modification.

EXPERIMENTAL

Cellulose regenerated by the viscose process (CRV®) was a generous gift from Hexabio (Bordeaux, France). All chemicals were of research grade purity and used without further purification.

Chemical Modification

Synthesis of Phosphorylated Cellulose

Regenerated cellulose (CRV) discs were machined from blocks dried in air at room temperature. The obtained CRV discs (10 × 2 mm) were swollen consecutively in distilled water, ethanol, and hexanol, for 24 h, prior to chemical modification. The phosphorylation reaction was carried out in a four-neck round-bottom flask equipped with a nitrogen inlet, a condenser, a thermometer, and a mechanical stirrer. CRV discs were dispersed in 30 mL of hexanol and a solution of phosphorus pentaoxide (50 g) in 37 mL of triethyl phosphate and 42 mL of 85% phosphoric acid was added portionwise to the suspension. The reaction was allowed to proceed at room temperature, under constant stirring and a nitrogen stream, for 4 h (P4), 8 h (P8), and 24 h (P24). Phosphorylated CRV (CRV-P) thus obtained was rinsed with hexanol and ethanol, washed by Soxhlet extraction, and then dialyzed against distilled and

deionized water, at least for 24 h, to wash out the excess H_3PO_4, until tests for inorganic phosphate were negative. The modified materials were then dried in air at room temperature.

Preparation of the Calcium Salt

The cellulose phosphate calcium salt was prepared by immersing each sample of the phosphorylated materials in 50 mL of a 0.05*M* $CaCl_2$ aqueous solution for 24 h at 37°C.

Characterization

Spectroscopic Analyses

X-ray photoelectron spectroscopy (XPS) analyses were performed on modified and unmodified cellulose discs using an ESCALAB 220i-XL spectrometer (VG Scientific, West Sussex, UK). Spectra from 0 to 700 eV were recorded using the nonmonochromatic MgK_α source at 200 W power. All binding energies were referred to the carbon 1s component, set to 284.6 eV. The XPS data were used to determine the chemical composition of the surface at about 5 nm depth and the chemical state of P and Ca. Surface etching was performed softly to eliminate surface contamination and carry out peak fitting without degrading the chemical bonding. Fourier transform infrared spectroscopy (FTIR) spectra were recorded on a Perkin–Elmer Model 137B Infracord spectrometer (Perkin Elmer Cetus Instruments, Norwalk, CT). Samples for analysis were scratched from the surface and prepared as dispersions in spectroscopic-grade potassium bromide (KBr). Solid-state ^{31}P MAS NMR spectra were recorded at 9.4 T with a Bruker DPX-400 NMR spectrometer (Bruker Instruments, Billerica, MA) operating at 161.97 MHz, using the Bruker's microprogram zg, with the following parameters: 1000 scans, a 6.5-μs pulse, with a delay time of 5.0 s, 31 ms of acquisition time, and a sweep width of 400 ppm. Studies were carried out at room temperature with zirconia rotors, using magic angle spinning rates of 8 kHz. Chemical shifts were determined using 85% H_3PO_4 (δ_{31P} = 0 ppm) as an external reference.

Roughness

Surface roughness of the original cellulose and the modified materials was characterized using a laser rugosimeter, model Perthometer S3P, from Perthen (Germany). Each value was the mean of five measurements.

Hydrophilicity

To determine surface hydrophilicity, contact angle measurements were carried out on modified and unmodified cellulose samples by the sessile drop method, at room temperature, using an optical bench-type contact angle goniometer (Model G23M; Krüss, Hamburg, Germany). Samples were previously dried at 23°C and 50% relative humidity, until constant weight was achieved. Four replicas of each sample were used and at least two drops of approximately 1 μL of deionized water, ethylene glycol, and diiodomethane were deposited on each disc, and the static contact angle was measured for each drop.

Morphology

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) analyses were carried out at 15 kV using an S-2500 scanning electron microscope (Hitachi, Tokyo, Japan). Observations were processed from sputtered Pd–coated specimens.

Sterilization Resistance

After packing, samples were gamma-irradiated for 8.5 h at room temperature, in both the presence and the absence of oxygen (using nitrogen), in a CIS Bio-Industries equipment, model IBL 337 (Gif-sur-Yvette, France), loaded with 13,000 Ci of ^{137}Cs, to absorb a total dose equal to 25 kGy. Resistance to sterilization by gamma-radiation was assessed by FTIR spectroscopy.

Water Swelling

CRV discs were dried in vacuum at 50°C until constant weight was attained. They were then soaked in water, at room temperature, and their wet mass was weighed at consecutive periods of time, until equilibrium was reached.

RESULTS AND DISCUSSION

As previously reported, when this phosphorylation technique was applied to modify microcrystalline cellulose, the swelling pretreatment significantly influenced the resulting products. An adequate swelling pretreatment promoted higher

modification yieldings by enhancing the accessibility of the materials structure.[18] In Figure 1 the behavior of regenerated cellulose after immersion in water, ethyl alcohol, and hexanol is shown. Water is one of the best cellulose swelling agents[21,22] but traces of water are deleterious to the present reaction.[18–20] Therefore, water must be exchanged before the reaction. However, as shown in Figure 1(a)–(c), water must be exchanged consecutively by ethanol and then hexanol, or otherwise the material cracks. Hence, on one hand, water was exchanged by ethanol, where it is soluble. Ethanol, on the other hand, is soluble in hexanol and was washed by the latter since hexanol is the solvent of the reaction itself. Hexanol is not a good swelling agent for cellulose but the material kept its swelling in water after the exchange in the sequence of ethanol followed by hexanol.

The calcium salt of cellulose phosphate was prepared because this is the composition under investigation for orthopedic applications. Previous results showed that the calcium salt mineralizes to a much higher extent than the materials that were only phosphorylated.[23] Figure 2 indicates the surface chemical composition of the calcium salt of phosphorylated cellulose hydrogels, obtained by XPS, where it can be seen that with increasing phosphorylation reaction time, %C decreased as %O, %Ca, and %P increased. Table I shows O/C, P/C, and Ca/C ratios obtained from XPS data. The decrease in carbon atomic percentage and the increase in that of oxygen, with increasing reaction times, seem to be in accordance with the achievement of surface phosphorylation, given that for each phosphate group grafted, one free hydroxyl on the original cellulose is substituted by two new hydroxyl groups and one phosphoryl group; hence, the oxygen content is increased. The XPS survey scan spectra (Fig. 3) show that, on unmodified cellulose, only C1s and O1s peaks are present. In addition, on phosphorylated cellulose, phosphorus and calcium were found. The intensity of phosphorus peaks increased with reaction time. As shown in Figure 2, after 4- and 24-h treatments, %P and %Ca increased from about 5 to 11, and from about 3 to 10, respectively. P/C and Ca/C ratios evolved following the same pattern (Table I). Peak fitting was carried out after analyses at low depth so as not to degrade the chemical bonding. The P2p-fitted spectrum can be seen in Figure 4. Phosphorus species were found at a binding energy of 133.7 eV. Furthermore, XPS data showed that P2p and Ca2p (347.4 eV) positions (the most prominent peaks that do not exist on unmodified cellulose) remained unchanged, independently of the phosphorus content, and the relative separation between their binding energies was approximately the same as that found for calcium phosphates.[24–27] This fact indicates that the chemical modification is homogeneous for varying reaction times. Binding energy positions for P2p were in the range usually found for calcium phosphates, that is, hydroxyapatite (the mineral of bone).[24–27] Similarly to these structures, in the present case, the oxygens of the phosphate hydroxyl groups are probably chelated by ionic bonds to the calcium ions.

EDS microanalysis was carried out along the diameter of surface-phosphorylated discs, using a magnification of $\times$1000, and the atomic percentage of phosphorus was determined, as shown in Figure 5. It can be seen that the reaction is homogeneous along the surface in that low standard deviations were obtained using different areas of independent samples. However, EDS data cannot be compared to XPS data because, in the former, data correspond to a much thicker layer and not only to the outermost one. Results obtained by EDS must be considered semiquantitative. The depth of phosphorylation also increased with increasing reaction time, as can be seen in Figure 6. Phosphorus depth profiles were obtained by EDS microanalysis, along a cross section, as a function of reaction time.

An ideal solid surface would be homogeneous in molecular composition and smooth at the molecular level.[28–32] However, real surfaces are heterogeneous, as well as appreciably rough and complex in topography. Contact angle measurements are usually the means to determine surface free energy of solids, recurring to the Young equation, and assuming that the surface is appreciably close to an ideal one. Roughness is among the factors that must be considered before using the Young equation and the relationship established therein. The effect of roughness on contact angles has been widely recognized to affect the contact angle and hence surface free energy.[5,28–32] The influence of biomaterials' surface roughness on their functionality has been demonstrated as well in a number of independent studies, pointing out the relevance of knowing the roughness of a candidate material for biomedical applications.[5,33–36] Laser rugosimetry measurements were then car-

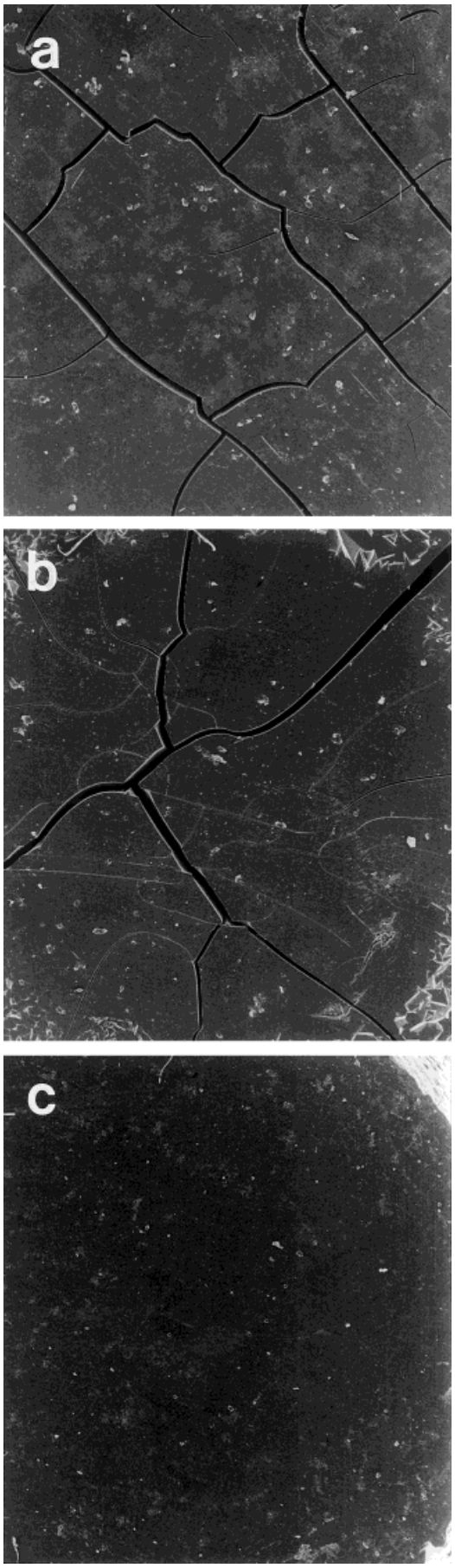

Figure 1 SEM micrographs of the surface of unmodified regenerated cellulose hydrogels (CRV) dried in air, after immersion in: (a) hexanol, (b) ethanol followed by hexanol, and (c) water, followed by ethanol and finally hexanol.

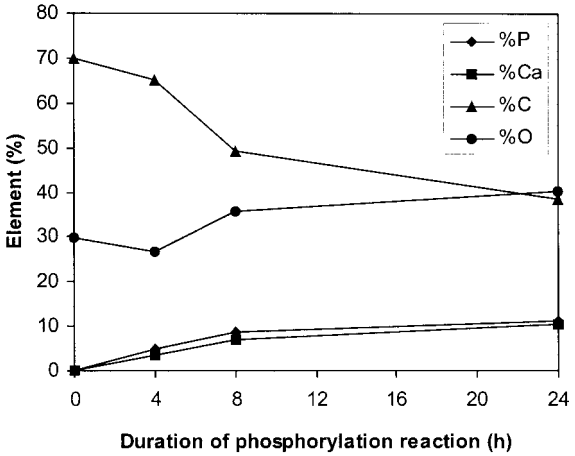

Figure 2 P, Ca, O, and C atomic percentages of unmodified and phosphorylated cellulose, obtained by XPS, plotted as a function of the phosphorylation reaction time.

ried out to determine the surface roughness of prepared sample discs (Fig. 7). It was shown that prepared surfaces are significantly rough. Discs were cut from cast cylinders and the roughness found reflected the effect of the cutting operation. Samples were not subsequently polished so as to avoid contamination of the surface. The phosphorylation reaction roughened the surface, although no significant differences were found as a function of reaction time.

As a consequence of the rough nature of prepared surfaces, surface free energies were not determined. In the present work, the main purpose of contact angle measurements was to assess comparative changes in hydrophilicity resulting from surface phosphorylation. Contact angle measurements confirmed the moderate hydrophilicity of regenerated cellulose hydrogels obtained by the viscose process. The relatively low contact angle ($\sim 32°$) in water obtained for unmodified regenerated cellulose was further decreased after phosphorylation (4 h) and higher phosphate contents further decreased it, although difficult-to-measure low angles were obtained for 8- and 24-h treatments (Fig. 8). A similar behavior was found using ethylene glycol, which is another polar liquid. When an apolar liquid was used (i.e., diiodomethane) the initial contact angle remained unchanged, according to the fact that polar solids

Table I Surface Chemical Composition of Untreated (CRV) and Phosphorylated Cellulose (CRV-P), at Varying Reaction Times, Obtained by XPS

	Element/C Ratio		
Material	O/C	P/C	Ca/C
CRV	0.43	0	0
CRV-P4	0.41	0.08	0.05
CRV-P8	0.73	0.17	0.14
CRV-P24	1.04	0.28	0.27

do not influence apolar liquids.[28] These values clearly demonstrated the high hydrophilic nature of cellulose phosphates but must be analyzed carefully. Surface roughness strongly influences the drop contact angle and, for rough surfaces (as it is the case in here), the Young equation is no longer valid.[28–32] Results are nevertheless comparatively valid but should not be considered as absolute. To obtain more precise data, dynamic contact angle measurements on smoother surfaces must be carried out and roughness taken into account.

Although CRV swells considerably in water, this material does not regain its total water-sorption capacity after drying. After drying the first time, CRV swells up to approximately 60 wt % (Fig. 9) and this value remains unchanged for further dryings. This behavior is probably the result of the loss of structural water on the first drying. Although regenerated cellulose is not a thermoplastic polymer, the role of water in this material is similar to that of a plasticizer, that is, when the material is dried it is no longer comparable to a hydrated elastomer but, rather, becomes comparable to a brittle material. OH groups bound to water molecules through hydrogen bonds and free or weakly bound water are also present. Phosphorylated cellulose gels absorbed considerably higher amounts of water compared to nonmodified cellulose, which was confirmed when phosphorylation was achieved in the bulk modification of microcrystalline cellulose.[18] In the present case, phosphorylation is restricted to the surface layer of regenerated cellulose and thus the effect of increased water-sorption ability was hardly noticeable, in absolute values. Nevertheless, a small increase in total water sorption was observed for phosphorylated materials (Fig. 9). The kinetics of absorption was also accelerated for phos-

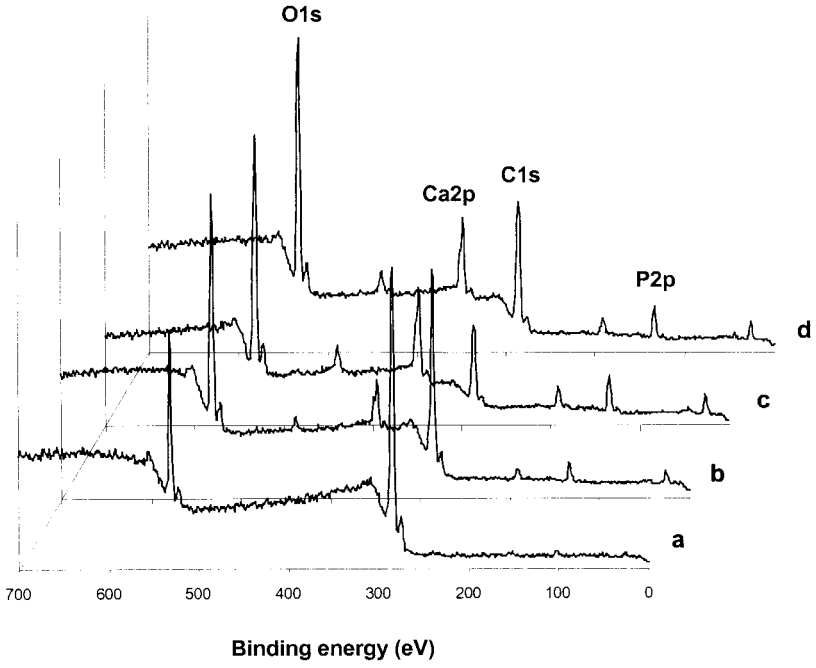

Figure 3 XPS survey spectra of unmodified and phosphorylated materials. (a) CRV; phosphorylated cellulose (CRV-P) at different reaction times: (b) 4 h, (c) 8 h, and (d) 24 h.

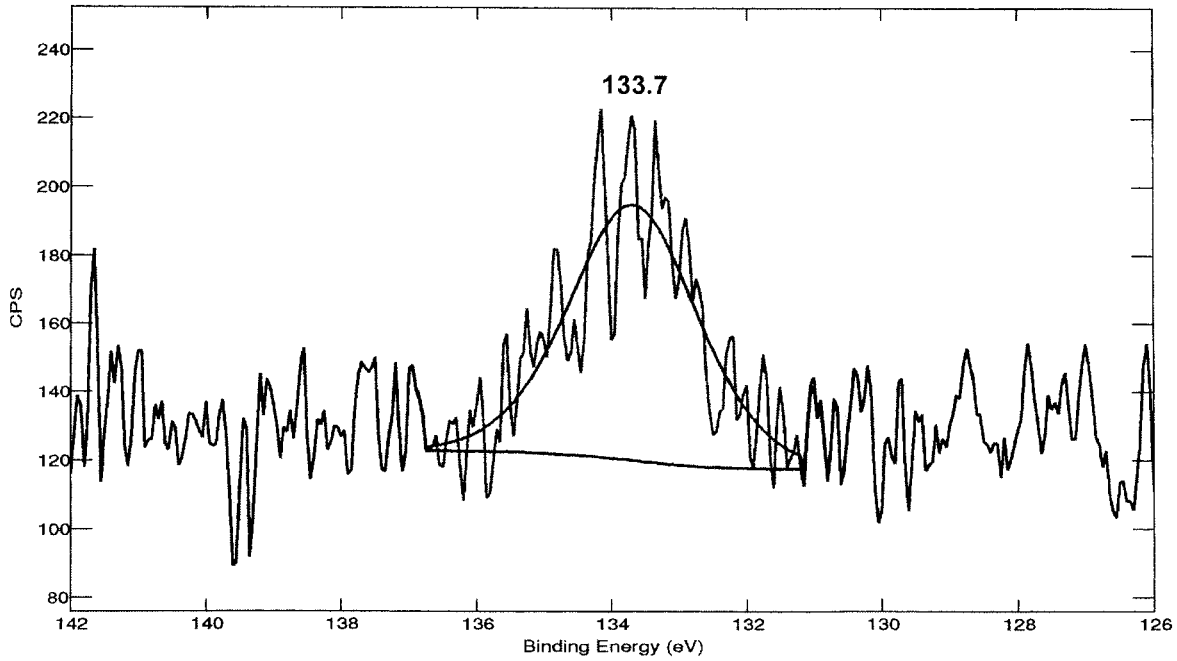

Figure 4 XPS high-resolution spectra of the P2p position on phosphorylated cellulose.

phorylated materials, the highest rates of which were found in the materials with the highest phosphate contents. Water sorption of CRV is closely related to its amorphous regions. Probably, surface phosphorylation promoted a slight increase in the amorphous-to-crystalline ratio, thus improving its water sorption. However, we demonstrated previously that the increase in water sorption should not be attributed only to a decrease in crystallinity, given that the water swelling of phosphorylated materials was higher than that of amorphous cellulose.[18]

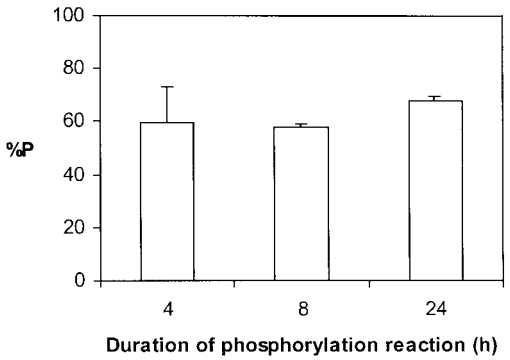

Figure 5 EDS %P data obtained along the diameter of surface phosphorylated cellulose, as a function of reaction time.

After incubation in phosphate-buffered saline (PBS), no considerable effect of phosphorylation on pH variation was observed, compared to that of unmodified regenerated cellulose (Fig. 10). Increasing phosphate contents promoted a slightly lower initial pH, which after 3 days, attained equilibrium pH (7.4). Because CRV-P is an acidic material, due to phosphate functionalities, its presence in a biological environment could be deleterious if not readily neutralized.

The FTIR spectra of phosphorylated samples (Fig. 11) showed the presence of a phosphate ester. A sharp peak at 1383 cm^{-1} can be attributed to P═O stretching.[37–39] Smoothing of the free OH groups at 3200–3600 cm^{-1} and C—H groups at 2950–2850 cm^{-1} was also observed, which can be associated with a decrease in crystallinity. A sharp peak at 1750 cm^{-1} was also present on unmodified cellulose, which is generally assigned to the carbonyl band (C═O),[40] indicating oxidation to some extent. In addition to the functional group absorptions, a peak was observed at 2350 cm^{-1}, which is attributed to the C—O stretch of carbon dioxide in the background.

Sterilization is a critical step in the validation of a medical device,[41] and gamma-radiation is generally designated as the most adequate method for cellulose sterilization, although chain scission may

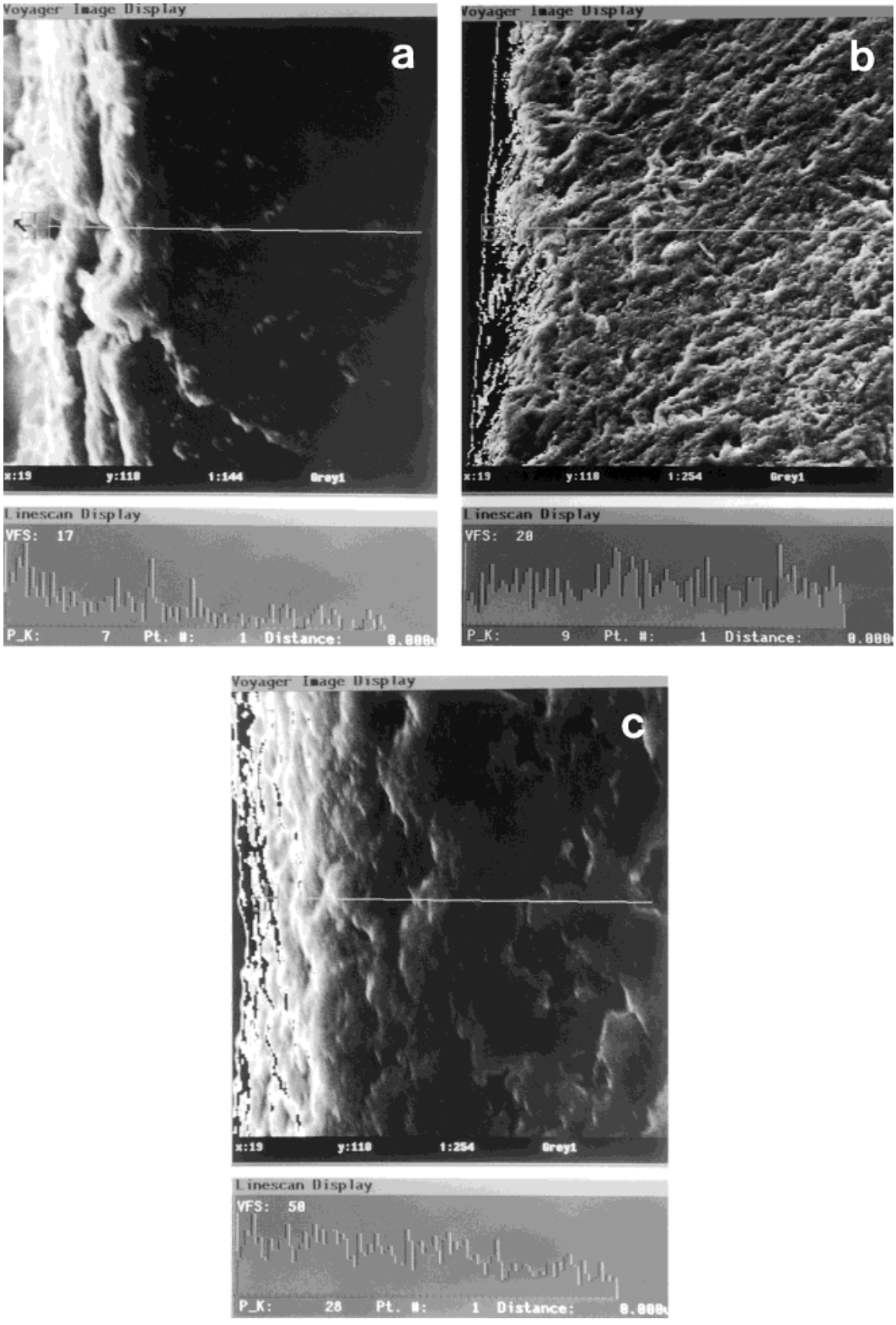

Figure 6 Depth of phosphorylation as a function of reaction time, as determined by EDS with image analysis techniques: (a) CRV-P4, (b) CRV-P8, and (c) CRV-P24. [color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

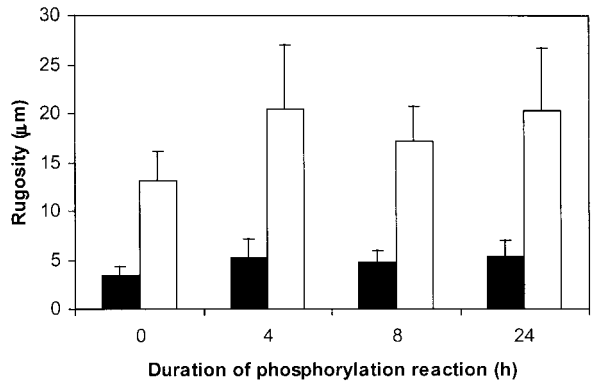

Figure 7 Roughness of unmodified and phosphorylated cellulose at different reaction times. R_a (black), mean roughness; R_z (white), roughness depth (mean peak-to-valley height).

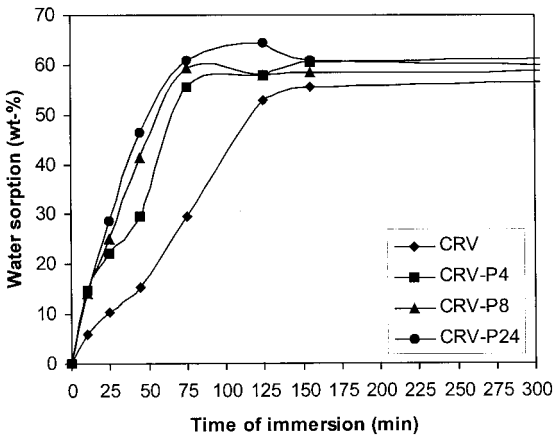

Figure 9 Swelling of CRV and CRV-P at different reaction times: 4, 8, and 24 h.

occur.[42–44] The effect of gamma sterilization on materials structure was assessed by FTIR spectroscopy on powdered samples scratched from the surface (Fig. 12). It can be seem that gamma sterilization did not promote structural changes, detectable by FTIR, on unmodified or phosphorylated cellulose, when a protected atmosphere was used (nitrogen). On the contrary, an oxidizing atmosphere promoted oxidation of the surface, as was demonstrated by the presence of carbonyl bands found at 1750 cm^{-1} in materials sterilized in air.

The ^{31}P MAS NMR spectrum of CRV-P (Fig. 13) exhibited a broad signal at 4.6 ppm. This chemical shift value is generally expected for ^{31}P in phosphate functionalities,[45–48] which, in conjunction with previous evidence, confirmed that phosphate groups are chemically bound to the material. MAS rate variation showed that symmetrical side bands were rotational bands resulting from powder anisotropy. The small sharp peak observed at −7.0 ppm indicates that more than one type of phosphate group is present in phosphorylated CRV. When ^{31}P MAS NMR was used to characterize phosphorylated cellulose gels obtained by chemical modification of microcrystalline cellulose,[18] this peak was not observed, suggesting that it may be ascribed to the interaction of phosphate ions with residues present in CRV's structure. Yokogawa et al.[49] recently reported similar findings when phosphorylating chitin fibers by the urea/H_3PO_4 method. In that

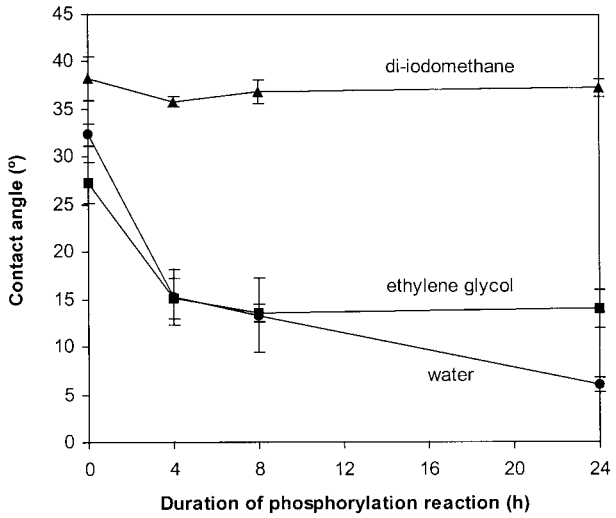

Figure 8 Contact angles of CRV ($t = 0$) and CRV-P, as a function of duration of the phosphorylation reaction, using liquids with different polarity: water (circles), ethylene glycol (squares), and diiodomethane (triangles).

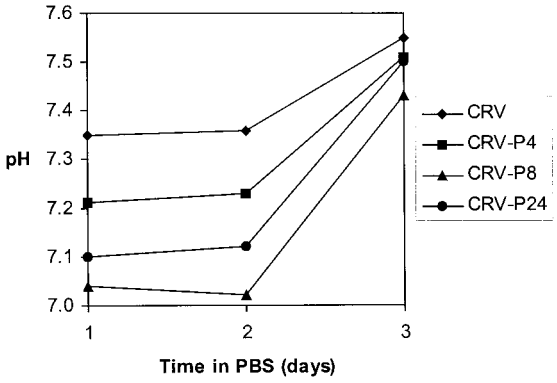

Figure 10 pH variation as a function of time of immersion of CRV and CRV-P in phosphate-buffered saline (PBS, without calcium and magnesium).

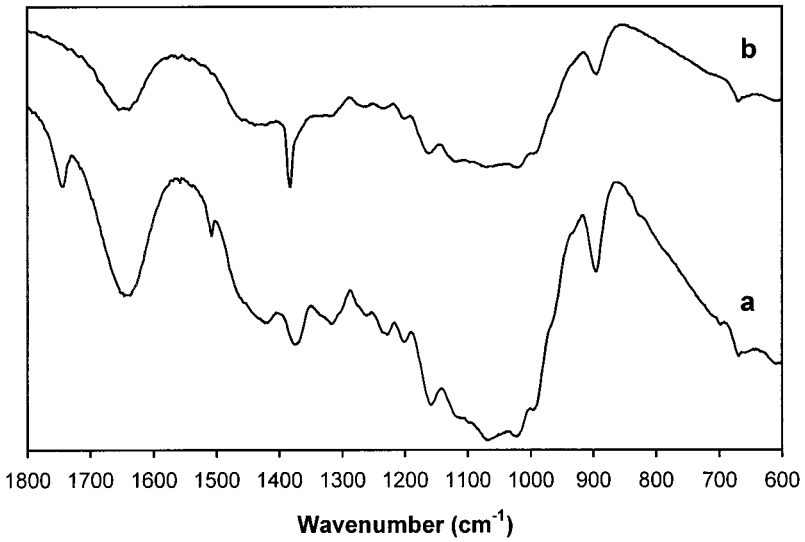

Figure 11 FTIR spectra of (a) CRV and (b) CRV-P8.

case, it was speculated that the second small peak, found at −9.9 ppm, was attributable to the ammonium hydrogen phosphate formed.[49] In the present case, ammonium-containing chemicals were not used.

CONCLUSIONS

The surface modification of regenerated cellulose hydrogels (CRV) was successfully carried out by phosphorylation, homogeneously, as demonstrated by XPS, FTIR, EDS, and ^{31}P MAS NMR spectroscopic analyses. Surface chemical modification by phosphorylation was optimized in terms of swelling pretreatment. The moderate hydrophilicity, determined by contact angle measurements, as well as water sorption of CRV were further increased through phosphorylation, and those effects were directly dependent on the phosphate content. Phosphorylation increased surface roughness but did not affect the sterilization re-

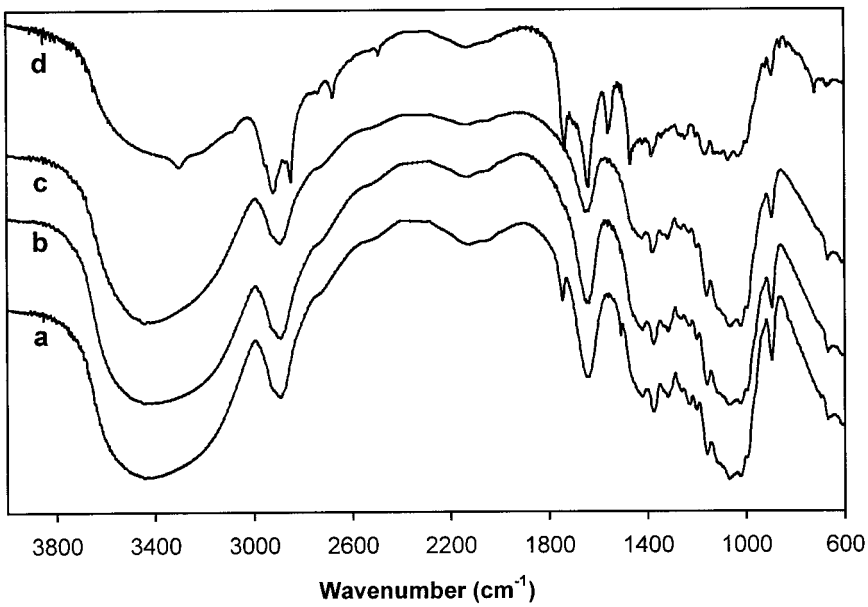

Figure 12 FTIR spectra of gamma-sterilized samples: (a) CRV; (b) CRV-P; CRV-P sterilized in (c) nitrogen atmosphere and (d) oxidizing atmosphere.

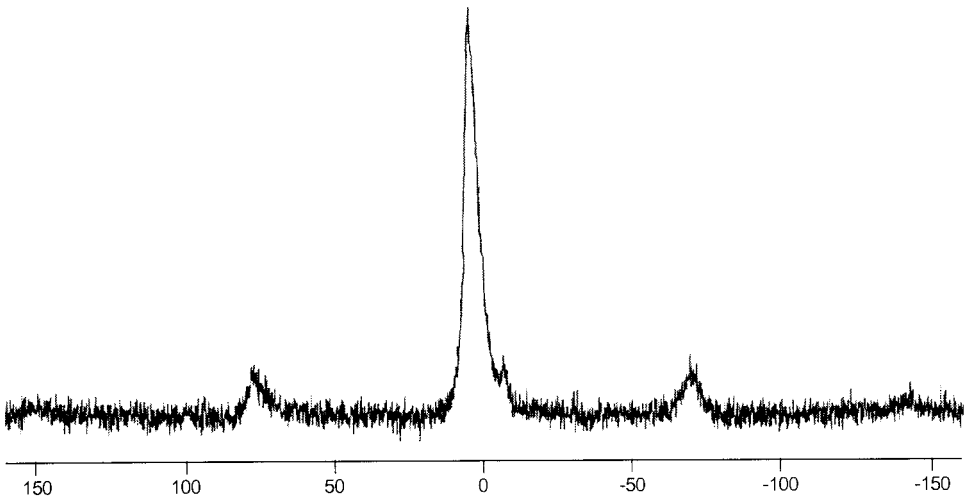

Figure 13 Solid-state ^{31}P MAS spectrum of CRV-P4.

sistance of CRV, if a nitrogen atmosphere was used. The high ability of phosphorylated cellulose to bind calcium ions was also demonstrated. Chemical bonding between calcium and phosphate functionalities resembled that usually found in calcium phosphates present in bone tissue, as demonstrated by XPS data.

Regenerated cellulose hydrogels have some physicochemical properties that make them adequate materials for biomedical applications, that is, biocompatibility associated with moderated hydrophilicity and water sorption similar to that of living tissues. Surface modification via phosphorylation endows CRV cellulose with different characteristics that can be used satisfactorily for biomedical applications, such as functionalization through grafting of the negatively charged and reactive phosphate groups, as well as cation exchange ability, high hydrophilicity, and water sorption.

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REFERENCES

1. Williams, D. F. Med Dev Technol 1995, 6, 6.
2. Gebelein, C. G. Biomimetic Polymers; Plenum Press: New York, 1990.
3. Hoffman, A. S. in Biomaterials Science: An Introduction to Materials in Medicine; Ratner, B. D.; Hoffman, A. S.; Schoen, F. J.; Lemons, J. F., Eds.; Academic Press: San Diego, 1996; Chapter 2, p. 124.
4. Kasemo, B.; Lausmaa, J. Environ Health Perspect 1995, 102, 41.
5. Ratner, B. D. J Biomed Mater Res 1980, 14, 665.
6. Poustis, J.; Baquey, C.; Chauveaux, D. Clin Mater 1994, 16, 119.
7. Gross, U.; Müller–Mai, C.; Voigt, C. in The Tissue Response on Cellulose Cylinders After Implantation in the Distal Femur of Rabbits, Proceedings of the 4th World Biomaterials Congress, Berlin, Germany, April 1992; p. 192.
8. Baquey, C.; Barbié, C.; More, N.; Rouais, F.; Poustis, J.; Chauveaux, D. in In Vivo Study of the Biostability of a Cellulose Material, Proceedings of the 4th World Biomaterials Congress, Berlin, Germany, April 1992; p. 365.
9. Chauveaux, D.; Barbié, C.; Barthe, X.; Baquey, C.; Poustis, J. Clin Mater 1990, 5, 251.
10. Pommier, J. C.; Poustis, J.; Baquey, C.; Chauveaux, D. Fr. Demande 8610331, 1986; Eur. Pat. 0256906 A1, 1987; U.S. Pat. 4,904,258, 1990.
11. Imbert, E.; Letourneur, D.; Jozefowicz, M. J Biomed Mater Res 1997, 34, 417.
12. Dowd, V.; Yon, R. J. J Chromatogr 1992, 627, 145.
13. Kennedy, J. F.; Cabral, J. M. S. in Solid Phase Biochemistry: Analytical Synthetic Aspects; Scoutch, W. H., Ed.; Wiley: New York, 1983; Chapter 7, p. 253.

14. Sugihara, J.; Imamura, T.; Yanase, T. J Chromatogr 1982, 229, 193.
15. Shih, T. Y.; Martin, M. A. Biochemistry 1974, 13, 3411.
16. Holmquist, W. R.; Schroeder, W. A. J Chromatogr 1967, 26, 465.
17. Thomaidis, T. S.; Fidalgo, B. V.; Harshman, S.; Najjar, V. A. Biochemistry 1967, 6, 3369.
18. Granja, P. L.; Pouységu, L.; Pétraud, M.; De Jéso, B.; Baquey, C.; Barbosa, M. A. J Appl Polym Sci 2001, 82, 3341.
19. Granja, P. L.; Barbosa, M. A.; Pouységu, L.; De Jéso, B.; Baquey, C. in Frontiers in Biomedical Polymers Applications, Vol. 2; Ottenbrite, R., Ed.; Technomic Press: Lancaster, PA, 1999; p. 195.
20. Granja, P. L.; Baquey, C.; Pouységu, L.; Bareille, R.; De Jéso, B.; Barbosa, M. A. Presented at Polymers in Medicine and Surgery, PIMS'96, Glasgow, UK, 1996; p. 73.
21. Klemm, D.; Philipp, B.; Heinze, T.; Heinze, U.; Wagenknecht, W. Comprehensive Cellulose Chemistry: Fundamentals and Analytical Methods, Vol. 1; Wiley–VCH Verlag: Weinheim, Germany, 1998; Chapter 2, p. 9.
22. Zeronian, S. H. in Cellulose Chemistry and Its Applications; Nevell, T. P.; Zeronian, S. H., Eds.; Ellis Horwood: Chichester, UK, 1985; Chapter 6, p. 159.
23. Granja, P. L.; Pouységu, L.; De Jéso, B.; Rouais, F.; Baquey, C.; Barbosa, M. A. J Appl Polym Sci to appear.
24. Ratner, B. D.; Castner, D. in Surface Analysis: The Principal Techniques; Vickerman, J. C., Ed.; Wiley: Chichester, UK, 1997; Chapter 3, p. 43.
25. Dupraz, A.; Nguyen, T.-P.; Richard, M.; Daculsi, G.; Passuti, N. Biomaterials 1999, 20, 663.
26. Nguyen, T.-P.; Dupraz, A. J Biomater Sci Polym Ed 1996, 8, 141.
27. Hanawa, T.; Ota, M. Biomaterials 1991, 12, 767.
28. Morra, M.; Volpe, C. D. J Biomed Mater Res 1998, 42, 473.
29. Garbassi, F.; Morra, M.; Occhiello, E. in Polymer Surfaces: From Physics to Technology; Wiley: New York, 1994; Chapter 4, p. 161.
30. Vogler, E. A. in Surfactant Science Series 49 (Wettability); Marcel Dekker: New York, 1993; p. 183.
31. Good, R. J. J Adhes Sci Technol 1992, 6, 1269.
32. Good, R. J. in Surface and Colloid Science; Good, R. J.; Stromberg, R. R., Eds.; Plenum Press: New York, 1979; Vol. 11, p. 1.
33. von Recum, A. F.; Brown, C. E.; Shannon, C. E.; LaBerge, M. in Handbook of Biomaterials Evaluation: Scientific, Technical, and Clinical Testing of Implant Materials, 2nd ed.; von Recum, A. F., Ed.; Edwards Brothers: Ann Arbor, MI, 1999; Chapter 13, p. 227.
34. Boyan, B. D.; Batzer, R.; Kieswetter, K.; Liu, Y.; Cochran, D. L.; Szmuckler–Moncler, S.; Dean, D. D.; Schwartz, Z. J Biomed Mater Res 1998, 39, 77.
35. Richards, R. G. Injury 1996, 27, S-C38.
36. Brunette, D. M. in Surface Characterization of Biomaterials; Ratner, B. D., Ed.; Elsevier Science: Amsterdam, 1988; p. 203.
37. Kuptsov, A. H.; Zhizhin, G. N. Handbook of Fourier Transform Raman and Infrared Spectra of Polymers, Physical Series Data 45; Elsevier: Amsterdam, 1998.
38. Nyquist, R. A.; Craver, C. D. in The Coblentz Society Desk Book of Infrared Spectra; Craver, C. D., Ed.; The Coblentz Society: French Village, MO, 1977; p. 399.
39. Nakamoto, K. Infrared and Raman Spectra of Inorganic and Coordination Compounds, 3rd ed.; Wiley-Interscience: New York, 1977.
40. Klemm, D.; Philipp, B.; Heinze, T.; Heinze, U.; Wagenknecht, W. Comprehensive Cellulose Chemistry: Fundamentals and Analytical Methods, Vol. 1; Wiley–VCH Verlag: Weinheim, Germany, 1998; Chapter 3, p. 181.
41. Matthews, I. P.; Gibson, C.; Samuel, A. H. Clin Mater 1994, 15, 191.
42. Chamberlain, V. C.; Lambert, B.; Tang, F.-W. in Handbook of Biomaterials Evaluation: Scientific, Technical, and Clinical Testing of Implant Materials; von Recum, A. F., Ed.; Taylor & Francis: Philadelphia, 1999; Chapter 15, p. 253.
43. Phillips, G. O.; Arthur, J. C., Jr. in Cellulose Chemistry and Its Applications; Nevell, T. P.; Zeronian, S. H., Eds.; Ellis Horwood: Chichester, UK, 1985; Chapter 12, p. 290.
44. Ahmed, A. U.; Rapson, W. H. J Polym Sci Part A Polym Chem 1972, 10, 1945.
45. Canet, D. Nuclear Magnetic Resonance: Concepts and Methods; Wiley: Chichester, UK, 1996.
46. Vogel, J.; Russel, C.; Gunther, G.; Hartmann, P.; Vizethum, F.; Bergner, N. J Mater Sci Mater Med 1996, 7, 495.
47. Wu, Y.; Glimcher, M. J.; Rey, C.; Ackerman, J. L. J Mol Biol 1994, 244, 423.
48. Rothwell, W. P.; Waugh, J. S.; Yesinowski, J. P. J Am Chem Soc 1980, 102, 2637.
49. Yokogawa, Y.; Paz Reyes, J.; Mucalo, M. R.; Toriyama, M.; Kawamoto, Y.; Suzuki, T.; Nishizawa, K.; Nagata, F.; Kamayama, T. J Mater Sci Mater Med 1997, 8, 407.