JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **10** (1999) 641–648

Resorbable polyacrylic hydrogels derived from vitamin E and their application in the healing of tendons

M. A. PLASENCIA
Hospital Principe de Asturias, Alcalá de Henares, Madrid, Spain

C. ORTIZ, B. VÁZQUEZ, J. SAN ROMÁN*
Instituto de C. y. T. de Polímeros, CSIC, Juan de la Cierva, 3, 28006-Madrid, Spain

A. LÓPEZ-BRAVO
Hospital Provincial de Ávila, Ávila, Spain

A. LÓPEZ-ALONSO
Facultad de Medicina, Universidad de Alcalá de Henares, Madrid, Spain

A hydrogel containing vitamin E (α-tocopherol) was prepared by free radical polymerization of 2-hydroxyethyl methacrylate (HEMA) and α-tocopheryl methacrylate (VEMA), the latter being synthesized previously to its use. The hydrogel containing 20 wt % of VEMA showed equilibrium water content in the range of those of hydrogel networks, at any pH. The swelling of the hydrogel followed Fick's law, indicating that sorption of water molecules is controlled by diffusion, although the values of diffusion coefficients for the VEMA-containing hydrogel were lower than those of poly-HEMA in any medium. Surface characterization of the VEMA-containing hydrogel revealed a decrease in the surface energy of solid owing to a decrease of the polar component mainly. The application of finely powdered xerogel of HEMA–VEMA copolymer bearing 20 wt % of the vitamin E derivative gave a very fast and positive response showing an activated regeneration capacity, probably due to the stimulation of the cellular proliferation or the more plausible effect, the cellular protection associated to the antioxidant properties of the vitamin E residue.

1. Introduction

It is widely recognized that biological oxidation is closely correlated with pathology; ageing oxygen radical damage has been implicated in specific biological processes and pathologies such as inflammation, arthritis, cardiovascular diseases, pulmonary dysfunction in hemodialysed patients, systemic lupus erythematosus, mutagenesis and carcinogenesis, etc. [1–6]. Inflammation is generally defined as the reaction of vascularized living tissue to load injury. This process is characterized by the release of specific chemical mediators from plasma cells and injured tissue which, in a complex dynamic equilibrium, interact and provide a system of checks and balances regarding their respective activities and functions. In addition, chemical mediators are quickly inactivated or destroyed, suggesting that their action is local predominantly (at the site of inflammation). In general, the lysosomal proteases and oxygen-derived free radicals produce the most significant damage or injury [4, 6] and show clearly the relationship between polyunsaturated fats, biological oxidations, oxyradicals and tissue damage and the corresponding antioxidant mechanism. In particular, vitamins C and E act in concert upon radicals and peroxides to reduce them to alcohols [1, 7]. On the other hand, another function of vitamin E is the contribution to stabilize biological membranes by means of specific physicochemical interactions between the phytyl side-chain of α-tocopherol and the fatty acid chains of polyunsaturated fatty acids, particularly those derived from arachidonic acid [2, 8].

Recently, Shubert and co-workers [9] have described the improving of the biostability of polyurethane elastomers *in vivo* by the effect of vitamin E and they consider that the mechanism of increase biostability involves the quenching of free radicals produced during the respiratory burst of macrophages associated with the inflammatory response of the organism to the presence of foreign implants. However, from a chemical point of view, vitamin E is a highly hydrophobic or lipophilic molecule incompatible with the hydrated medium, but we have prepared recently a new acrylic monomer derived from vitamin E [10] that can be polymerized with hydrophilic acrylic or vinyl monomers to obtain hydrogels bearing vitamin E structures as side groups of the high-molecular-weight polymeric chains. The acrylic structure of this vitamin E derivative offers

*Author to whom correspondence should be addressed.

interesting opportunities for the design and preparation of biomaterials with specific properties according to applications. In this sense, polymeric systems based on hydrophilic acrylic monomers such as 2-hydroxyethyl methacrylate (HEMA), acrylic acid, acrylamide derivatives, etc., have found wide applications in medicine and surgery because of their ability to form biocompatible hydrogels with excellent tolerance and good stability. The copolymerization of HEMA with other selected vinyl and acrylic monomers provides an excellent way to prepare biomaterials with controlled hydrophilicity and specific functions depending on the hydrophobic character and chemical structure of the second monomer, the average composition and distribution of comonomeric units along the macromolecular chains, and the specific effect of the active side residues supported by the polymeric acrylic backbone.

In this paper, the synthesis and characterization of copolymers prepared by the free radical polymerization of HEMA and vitamin E methacrylate (VEMA) is presented. A parallel *in vivo* study of the application of finely powdered xerogel of HEMA–VEMA copolymer bearing 20 wt % of the vitamin E derivative was carried out. Histological analysis of the healing processes modulated by the presence of the HEMA–VEMA copolymer systems were examined and compared qualitatively.

2. Experimental procedure

2.1. Materials

Vitamin E (α-tocopherol) (Merck) was used as received without further purification. Methacryloyl chloride (Fluka A.G.) and triethylamine (Scharlau) were purified by distillation under reduced pressure. Azobisisobutironitrile (AIBN) (Merck) was recrystallized from methanol (melting point 104 °C). HEMA was purified according the literature [11]. The solvents diethyl ether (Quimicen), isopropanol (Quimicen) and dimethylformamide (Scharlau) were purified by standard procedures.

Fifty-seven New Zealand male rabbits of an average weight of 3 kg were used for *in vivo* experimentation. Twenty animals were used in control experiments and the rest in the implantation of the novel hydrogel, of which 20 animals were employed in immobilization experiments and 17 in mobilization experiments which started on the fifth day after implantation. Three tendons of each group were evaluated histologically after 3, 10, 20 and 30 days of the operation. Likewise, eight more animals of each group were sacrificed after 30 days of healing; two of them were examined by scanning electron microscopy and the other six were used for biomechanical tests.

2.2. Synthesis of α-tocopheryl methacrylate

α-Tocopherol (20 mmol), triethylamine (20 mmol) and diethylether (50 ml) were introduced into a three-necked flask and the methacryloyl chloride (20 mmol) was added dropwise with constant stirring at room temperature under nitrogen atmosphere. The reaction mixture was then stirred for 24 h at room temperature. The reaction medium was filtered to remove the triethylamine chlorhydrate and the unreacted reagents were removed by successive extraction with 5% Na OH solution. After drying over $MgSO_4$ the solvent was removed by flash distillation and then under reduced pressure until constant weight.

2.3. Preparation of hydrogels

Copolymerization reaction of VEMA and HEMA were carried out in solution of dimethylformamide (DMF) ($[M] = 1\,\mathrm{mol\,l^{-1}}$) at 50 °C using azobisisobutyronitrile (AIBN) as initiator ($1.5\,10^{-2}\,\mathrm{mol\,L^{-1}}$). Copolymers with 5, 10 and 20 wt % of VEMA were obtained at total conversion after 24 h of reaction. Copolymers were precipitated in diethyl ether, filtered off, washed and dried under vacuum until constant weight. Copolymer films were prepared by dissolving 2 g of the copolymer in 3 ml of a mixture of DMF/isopropanol. The solution was poured into a Teflon mould specially designed to provide a 4 cm diameter, 0.5 mm thickness disc. Solvent mixture was allowed to evaporate at room temperature during the first few hours and then under vacuum to ensure complete evaporation of the solvent.

2.4. Swelling behavior

Films accurately weighed were immersed in buffered solutions of pH = 3 (citrate/hydrochloride acid), pH = 7 (phosphate) and pH = 9 (boric acid/potassium chloride–sodium hydroxide) at room temperature. The water uptake was measured every 5 min in the first stages, until reaching the equilibrium, which was considered when three consecutive measurements gave the same weight. Water content (WC) was expressed in terms of weight relative to the wet weight (W)

$$\mathrm{WC} = (W - W_o)/W \tag{1}$$

W_o being the dry weight. Equilibrium water content (EWC) was WC determined when the sample had reached equilibrium.

2.5. Surface characterization

The contact angle measurements were performed on dry films of xerogels with a contact angle measuring system G10 (Krüss). The surface free energy was calculated by using the equations proposed by Owens [12]

$$(1 + \cos\theta)\gamma_l/2 = (\gamma_s^{\,d}\gamma_l^{\,d})^{1/2} + (\gamma_s^{\,p}\gamma_l^{\,p})^{1/2} \tag{2}$$

$$\gamma_s = \gamma_s^{\,d} + \gamma_s^{\,p} \tag{3}$$

where γ_s and γ_l are the surface free energies of the solid and liquid and $\gamma_s^{\,d}, \gamma_s^{\,p}, \gamma_l^{\,d}$ and $\gamma_l^{\,p}$ are the dispersion force components and polar force components of the surface free energy of the solid and the liquid, respectively. The liquids used for this purpose were methylene iodide and distilled water. The dispersion force component and the polar force component of the surface energy of water are 21 and $51\,\mathrm{mN\,m^{-1}}$ respectively, and the dispersion force component of the methylene iodine is $50\,\mathrm{mN\,m^{-1}}$.

2.6. Surgical procedure

The anaesthesia consisted of an i.m. injection of 3 cm^3 of a mixture of ketamine (35 mg kg^{-1}) xilacine (5 mg kg^{-1}) and acepronecine (2 mg kg^{-1}). A longitudinal cutaneous gap of approximately 3 cm was produced in the direction of the opening paratendon. Afterwards, the portion of the long plantar tendon, which shares the same coating capsule with the Achilles' tendon, was extracted in order to avoid adherence. Both fascicules of Achilles' tendon were cut transversely in a subtotal way at a constant distance of 2 cm from its insertion in the calcaneus. At that moment, the implantation of the hydrogel containing the vitamin E derivative was carried out between both tendon ends. No suture of the sectioned tendon was made to avoid interference in the healing process. On the other hand, a suture of Vicryl 5(0) was made in the paratendon in order to protect the hydrogel. Finally, the skin was sewn with discontinuous silk stitches 3(0) and the limb was immobilized leaving the ankle in planar flexion and the knee bent at 90°. During the anaesthetic induction and 48 h after the operation a doses of 0.5 ml of Dipenisol Retard (Bayer) was administered as antibiotic prophylaxis. This procedure was applied to every animal. In the mobilization experiments the immobilization was removed after the fifth day of the operation, and the animals were allowed to walk around in the cage.

2.7. Optical microscopy

Once the animals were sacrificed with a lethal injection of pentobarbital, the excised tendons were fixed in a buffer of 10% formaldehyde. Specimens were sectioned longitudinally and then embedded in paraffin. The microtome sections were stained with hematoxylin and eosin for cell composition and Masson's trichromic for organization and orientation of the collagenous extracellular matrix, to be viewed with polarized light microscopy.

2.8. Transmission electron microscopy

Two animals of each group were anaesthetised after 30 days of the initial lesion in order to carry out an *in situ* fixation of the specimen at the moment of its extraction. The extracted specimens were allowed to complete fixation in 0.1 cacodylate buffer 4% glutaraldehyde during 24 h. After washing the specimens in buffer solutions they were post-fixed with 1% osmiun tetroxide, and then they were dehydrated in ethanolic solutions of increasing concentrations. Clearing was carried out with propylene oxide and Araldite. Specimens were sectioned with an ultra microtome which provides cuts of 50 μm. Finally, the samples were stained with uracil acetate and Pb citrate. A transmission electronic microscope Zeiss EM 109 was used for this study.

2.9. Biomechanical study

Eighteen animals in total (six of each group) were sacrificed after 30 days of the operation and used for the biomechanical study. In these animals a resection of the Achilles' tendon together with the calcanei was performed. The diameter of each specimen was measured through the scar with an electronic calibration. After removing the fibrous tissue from the proximal tendon the specimens were isolated and stored at − 70 °C. Four hours before the biomechanical testing the specimens were thawed to room temperature, and maintained in serum. The analysis was performed in an Instron machine (Instron-MTS) operating with a cell load of 500-Newton and at a crosshead speed of 1 mm min^{-1}. Statistical analysis with Student's *t*-test was applied to the results ($\alpha = 0.05$).

3. Results and discussion

3.1. Preparation and characterization of the vitamin E-containing hydrogel

With the aim of taking advantage of the antioxidant properties of the vitamin E, an acrylic monomer containing this residue has been synthesized. The synthesis was carried out from α-tocopherol and methacryloyl chloride in the presence of triethylamine as catalyst. The product was isolated, purified and characterized by proton nuclear magnetic resonance (NMR) spectroscopy, as has been reported in a previous paper [10]. The concentration of VEMA in the feed was not greater than 20 wt % in order to have the adequate hydrophilic character of the copolymer system with good adhesion to the operated tendon. The reaction was carried out at total conversion so that an average copolymer composition similar to that of the feed was obtained. From a previous study on the copolymerization of this pair of monomers at low conversion the reactivity ratios were $r_{VEMA} = 0.72$ and $r_{HEMA} = 0.53$, indicating that statistical copolymers at any conversion are obtained. The sequence distribution in terms of triads of these copolymers was determined from the values of the reactivity ratios and is plotted in Fig. 1 versus the weight percentage of VEMA in the copolymer. It can be observed that in the range of 20 wt % VEMA in the copolymer, the units of the vitamin E derivative will be surrounded by units of HEMA, as is clearly shown in the figure. This means that there are not hydrophobic interactions between vitamin E neighboring units,

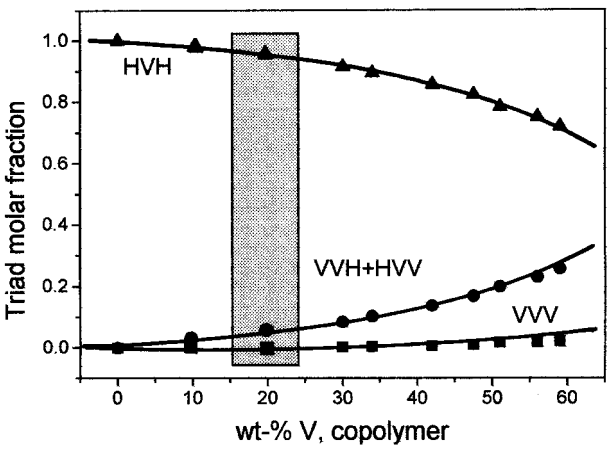

Figure 1 Variation of the molar fraction of the triad centred in VEMA with the copolymer composition.

which makes the interactions of these residues with proteins and cellular compounds of the surrounding tissues easier.

Swelling and surface characteristics of the new hydrogel were studied because of their importance in the prediction of the interaction of the hydrogel with the surrounding medium. First the 20 wt % VEMA hydrogel was submitted to swelling at different pHs, 3, 7 and 9. Fig. 2 shows the sorption of water with time of treatment for the novel hydrogel and for poly-HEMA. Water sorption was faster in the first stages either for the HEMA- or the VEMA-containing hydrogel, and after a relatively short period of time (approximately 100 min) the equilibrium was attained. The EWC for the VEMA hydrogel at any pH was lower than that of poly-HEMA, the biggest difference being for pH = 9. No sensitivity to pH was observed, as expected for non-ionizable polymers. However, a slight increase of EWC with pH was observed for poly-HEMA and a slight decrease for the VEMA containing hydrogel. Nevertheless, all the EWCs were higher than that considered as necessary to form a hydrogel network, indicating that the material will swell with physiological fluids once implanted in the human body.

Swelling kinetics were also studied. First, Fick's law [13] (Equation 4) was applied to the experimental data, that is, the reduced water sorption data were plotted versus the square root of the time, $t^{1/2}$, and initial rectilinear behavior was observed in all cases. From the slopes of the lines the value of the diffusion coefficients were obtained by using Equation 4, which can be applied for thin sheets in which diffusion through the edges may be neglected

$$M_t/M_\infty = 4(Dt/\pi l^2)^{1/2} = W_t/W_\infty \qquad (4)$$

M_t and M_∞ represent the water uptake at time t and at infinity, respectively, D is the diffusion coefficient and l is the average thickness of the film. The values obtained are plotted in Fig. 3. Lower values of D were obtained for the hydrogel containing vitamin E.

On the other hand, it is well established that there is the good biocompatibility of poly-HEMA hydrogels. This

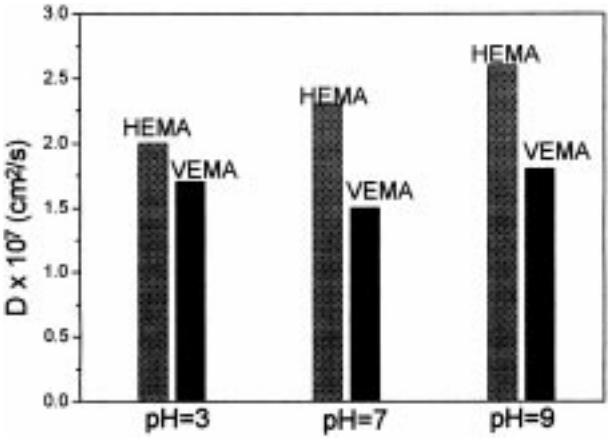

Figure 3 Values of diffusion coefficient according to Fick's law, for HEMA and 20 wt % VEMA containing hydrogel in different pHs.

polymer possesses a high surface energy with a strong polar contribution. This characteristic is considered to be positive in order to avoid protein adsorption which is one of the most common phenomenon that takes place when an implant biomaterial is put in contact with the physiological medium. The effect of the vitamin E residues of the new hydrogel on the surface properties was studied by means of contact angle measurements. It was observed that the presence of 20 wt % VEMA in the hydrogel gave rise to a decrease in the surface energy of solid mainly due to a decrease in the polar contribution (Fig. 4). It was also observed that this decrease was higher than that expected considering the average composition of the copolymer and the fractional contribution of each component, and it suggests the segregation of the components in microdomains [14], which would be consistent with the difference in polarity of both chemical structures and with the abundance of HHH sequences in the copolymer for this copolymer composition.

An important characteristic of the copolymer system with a content in the acrylic derivative of vitamin E lower than 25–30 wt % is that, in the experimental conditions of the present work, a linear microstructure of

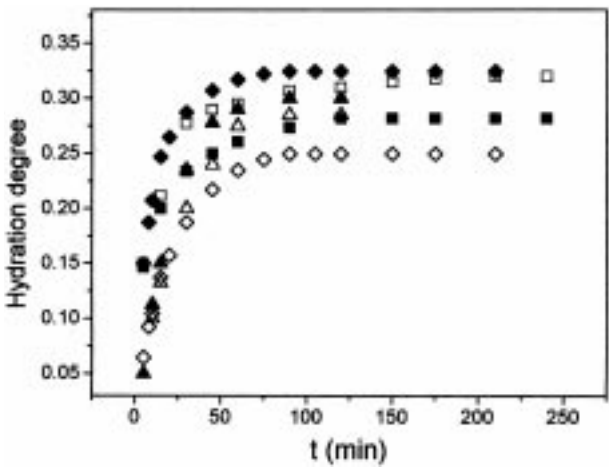

Figure 2 Swelling behavior of the hydrogels in different pHs: pH = 3: (□) HEMA and (■) VEMA hydrogel; pH = 7: (▲) HEMA and (△) VEMA hydrogel; pH = 9: (◇) HEMA and (◆) VEMA hydrogel.

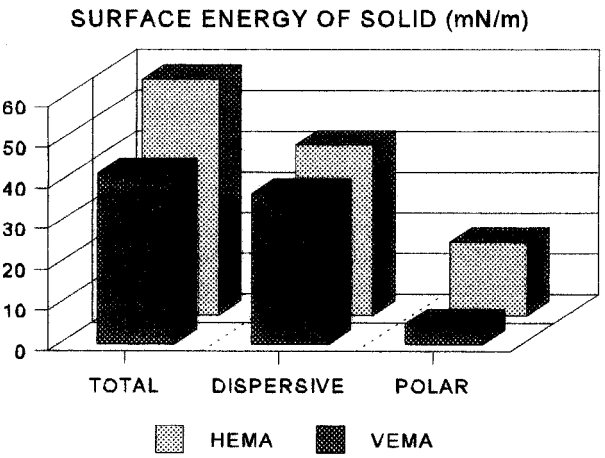

Figure 4 Values of the surface energy of solid and its dispersive and polar components for HEMA and 20 wt % VEMA containing xerogel.

the macromolecular system without chemical cross-linking between the copolymeric chains is obtained. In addition, the copolymer chains present an average molecular weight of $M_n = 35\,000$ Da. In these conditions the hydrogel does not have a tridimensional network structure and therefore, in the long term (e.g. several weeks) the copolymer mass is disintegrated, diluted and dissolved in the physiological fluids, which avoids the accumulation of the gel in the healed tissue and guarantees the clearance from the body of the system applied.

3.2. *In vivo* experimentation and histological analysis

As is well known, the reparative process of a tendon involves a scarce vascular support and the necessity of producing a great deal of collagen protein which will be remodelled subsequently. All this implies the immobilization of the operated limb at least for 6 or 8 weeks, with the associated side effects, such as muscle atrophy or articular rigidity, which gives rise to a slower recuperation. The aim of the *in vivo* experimentation was to analyze the influence of the hydrogel containing the vitamin E in the regeneration of Achilles' tendon. The methodology applied is represented schematically in Fig. 5. Particles of the copolymer hydrogel VEMA with an average size 50–250 μm were deposited directly on the surface of the sectioned tendon and the deposit was hydrated with two drops of saline solution. In a few minutes the powder particles were hydrated to give a gel with good adhesion to the surface of the sectioned tendon. The ends of the tendons were approximated and the position was kept with a suture point using resorbable vicryl 5(0). Different experiments in rabbits were carried out, in which the influence of the mobilization of the animals during the healing process was also studied. At first sight, a good remodeling of the tendons was obtained either for the control tendons or for those treated with the hydrogel. However, the latter showed a smoother surface with little adherence to the surrounding tissues. On the other hand, the tendons submitted to mobilization showed an increase in the distance between the two sectioned ends, but in no case was the fracture of the tendon observed.

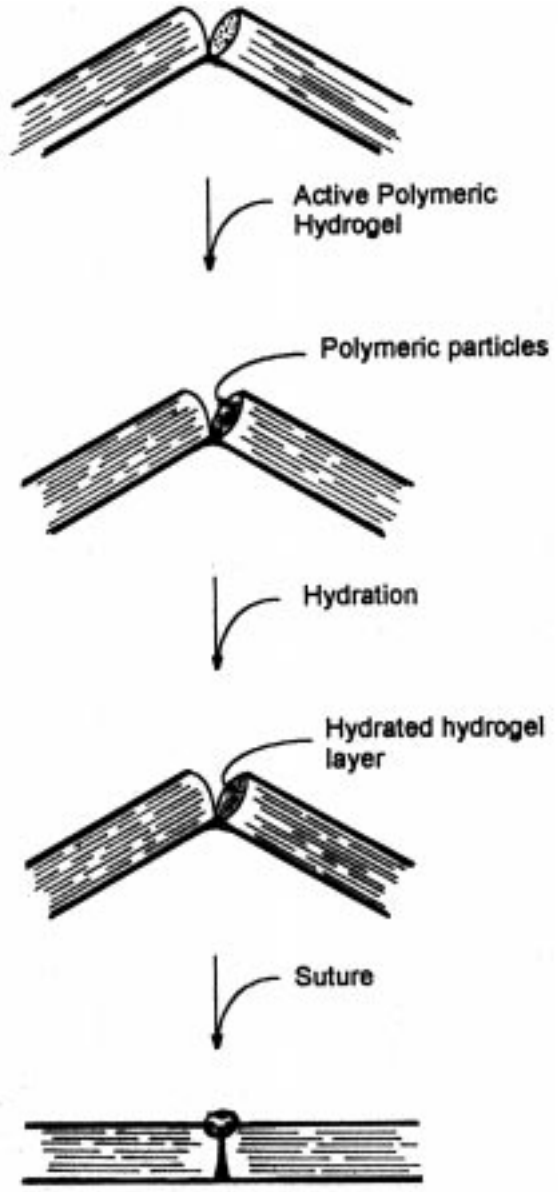

Figure 5 Schematic representation of the application of VEMA xerogels to excised Achilles' tendon.

As far as histology is concerned, after 3 days of the operation, the reparative area in the control experiments consisted of a not very well defined fibrin network where inflammatory and loose fibroblast-like cells were identified (Fig. 6a). However, in the treated tendons it was observed that the presence of the hydrogel allowed the organization of the loose fibrous network (Fig. 6b).

After 10 days, the reparative tissue in the control tendons was formed by loose and disorganized fibrovascular tissue which appeared to be orientated perpendicular to the axis of the tendon (Fig. 7). The healing tissue was growing without any orientation with respect to the direction of the tendon fascicule. A moderate number of fibroblasts and blood vessels perpendicular to the axis of the tendon were observed in the amplified micrograph of Fig. 7b. On the contrary, in the tendons treated with the vitamin E-containing hydrogel (Fig. 8) a greater fibroblast proliferation was observed, and the packed fibers appeared to be aligned in the axial direction of the tendon. In addition, these tendons showed regions of collagen bundles in the middle which were observed mainly in the middle of the scar in the tendons submitted to mobilization. The formation of a fibrovascularized tissue with fine bundles of neoformed collagen starting from the end of the excised tendon is observed clearly in Fig. 8a. Fig. 8b shows a number of fibroblasts in the vicinity of the original tendon with a clear longitudinal orientation following the axis of the tendon.

After 20 days, the fibrous network was replaced by a disorganized extracellular collagen matrix in the control tendons. Fibroblasts as well as some macrophages in the central area of the operated zone were the predominant cell. The reparative tissue of the treated tendons presented a noticeable superior vascular and cellular component. In the middle of the scar some residue of hydrogel could be observed, although the presence of

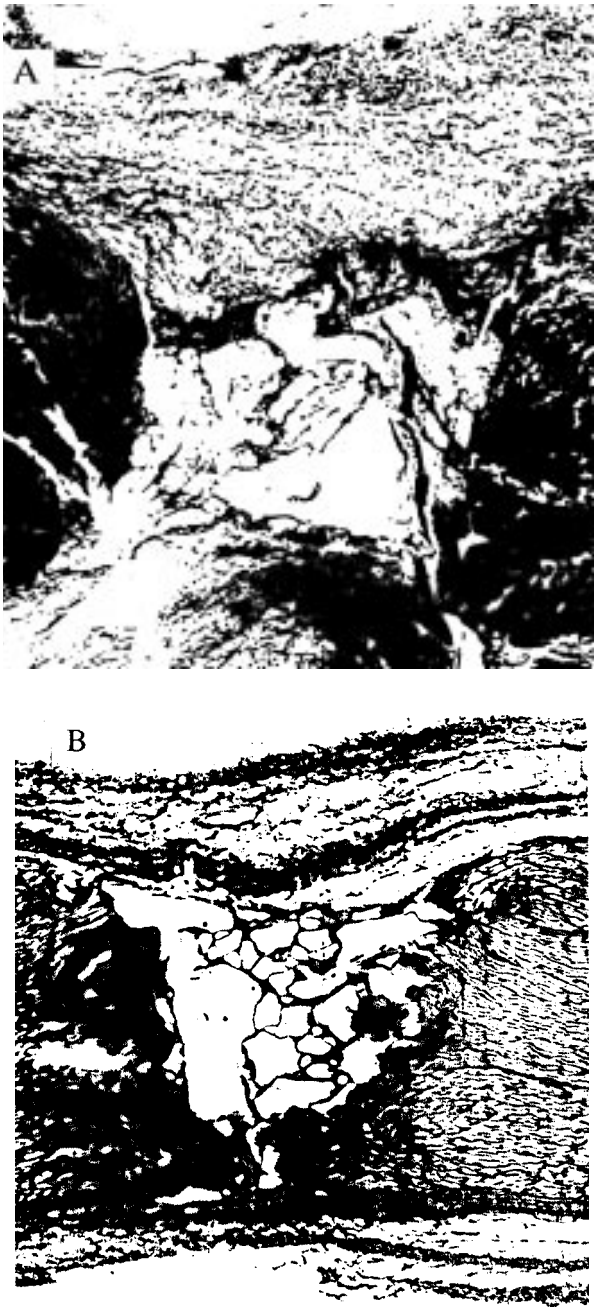

Figure 6 Optical microscopy of a cross-section of the regeneration zone after 3 days of surgical operation. Magnification 25×. (a) Control. (b) With the hydrogel of VEMA.

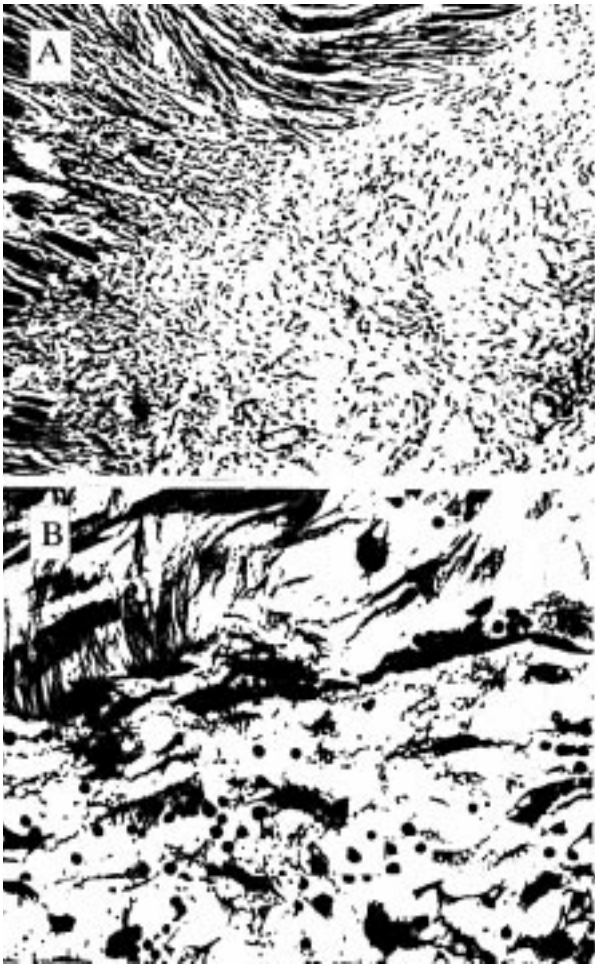

Figure 7 Optical microscopy of a cross-section of the regeneration zone proximal to the end of the excised tendon after 10 days of implantation stained with Masson's trichromic. Control group. (a) Magnification 25×. (b) Magnification 400×.

histiocytes or multinuclear cells, indicative of foreign body reaction, were not detected in these cases. In the tendons submitted to mobilization the neoformed collagen fiber appeared better aligned in the longitudinal direction of the tendon than in the other groups, and they were placed in the scar in a more uniform way. Fig. 9a corresponds to the operated zone with some residues of the polyacrylic derivative of vitamin E, distributed as isolated domains in a fibroblastic tissue with well-organized and orientated bundles of collagen fibers. The presence of red blood cells and blood vessels in the remaining hydrogel domains is observed clearly; some of them are indicated by black arrows in the right corner of the micrograph. Fig. 9b corresponds to an animal allowed to move with freedom, without immobilization, and in that case the bundles of collagen fibers are very well organized and orientated following the axis of the tendon. There is no sign of any foreign body reaction, without inflammatory cells.

Finally, after 30 days, either the control or the experimental tendons showed a good participation of the endotendinous cells in the reparative process and the presence of a number of blood vessels in the healing zone. A neoformed collagen fiber could be observed with a moderate organization of bundles (Fig. 10a). On the other hand, the tendons allowed to mobilize presented less vascularization and densely packed collagen bundles as a result of a good remodeling at the border of the sectioned tendon (Fig. 10b).

After the analysis of the samples through electron microscopy it can be said that the morphological characteristics of the fibroblast responsible for the reparative process of the tendon were independent of the group studied after 30 days of the lesion. A nucleus in interface, a well-developed endoplasmatic reticulum, a well-defined Golgi apparatus with many ribosomes and poliribosomes, were observed, all this being indicative of the good activity of the cell. The extracellular medium was formed by neoformed collagen fibers with an average diameter of approximately 30 μm. In particular areas of the cytoplasm in the tendons treated with the hydrogel, the presence of a variable number of collagen

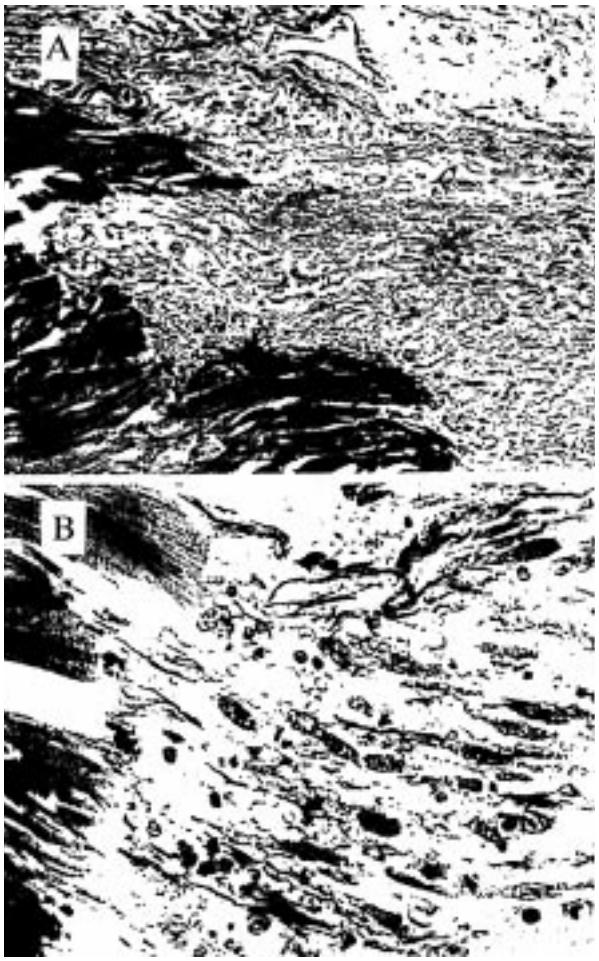

Figure 8 Optical microscopy of a cross-section of the regeneration zone proximal to the end of the excised tendon after 10 days of implantation. Staining with Masson's trichromic. Group treated with vitamin E derivative. (a) Magnification 25×. (b) Magnification 400×.

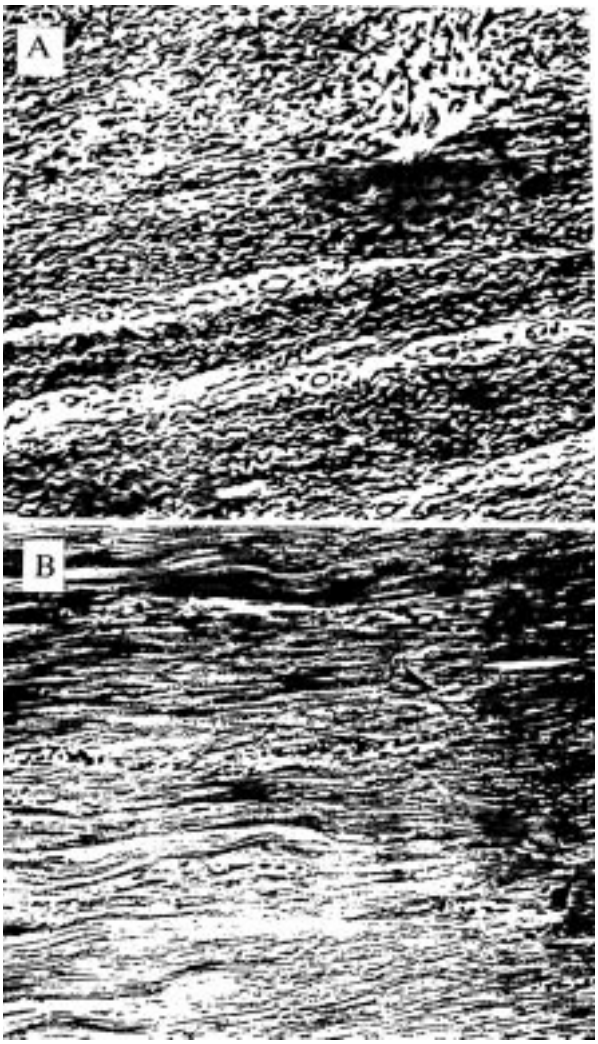

Figure 9 Optical microscopy of a cross-section of the regeneration zone after 20 days of surgical operation with the implant of hydrogel derived from vitamin E. Staining with Masson's trichromic (100×). (a) Group of immobilized rabbits. (b) Group of mobilized rabbits after the fourth day of operation.

fibers was detected. In these areas some microfilaments of 10 μm diameter, which appeared to be incorporated into the collagen fibers, were observed.

The tendons were also tested biomechanically. All the tendons failed through the reparative area except for two belonging to the experimental group, which failed through the osteotendinous union. The diameter of the tendon in the reparative area ranged between 4.6 and 7.8 mm with an average diameter of 6.3 mm and no significant differences were observed for the different groups. The highest stress to failure was obtained for the tendons submitted to mobilization $(83.5 \pm 12\,\text{MPa})$ in comparison to that obtained for the control $(78.8 \pm 5.9\,\text{MPa})$ and for the experimental group $(79.7 \pm 11.7\,\text{MPa})$, although no significant differences were observed among the different groups. The elongation to failure was in the range 1.5–1.8 mm for all the groups.

In conclusion, it can be said that the response of the tendon to the hydrogel was satisfactory. No necrosis or anomalous fibrosis, indicative of foreign body reaction, was detected. On the contrary, a good restorative response was observed, characterized by a more organized cellular response where the hydrogel was used as a support for the integration of fibroblasts and new collagen fiber; the tendons treated with the vitamin E hydrogel did not show an increase in the stress to failure in comparison with those of the control. The results are consistent with those studies performed with other macromolecules such as fibrin [15] or hyaluronic acid [16]. In spite of the fact that the mechanism of the regeneration generated by the hydrogel is still unknown, on the one hand, the hydrophilicity and therefore, the ability to absorb fluids, could play an important role. On the other hand, the antioxidant property of vitamin E could act, neutralizing the degradation products of phagocytosis and interfering in the inflammatory response in the healing tissue. This could favor the absence of adherences observed in the treated tendons. Also, recent studies have reported the ability of HEMA to reduce the formation of adherences when it was used as synthetic dural prosthesis [17].

Therefore, it can be said that this hydrogel could be an adequate system for the controlled release of specific growth factors, capable of accelerating the regeneration process and it could avoid the long immobilization involved in the healing of a broken tendon.

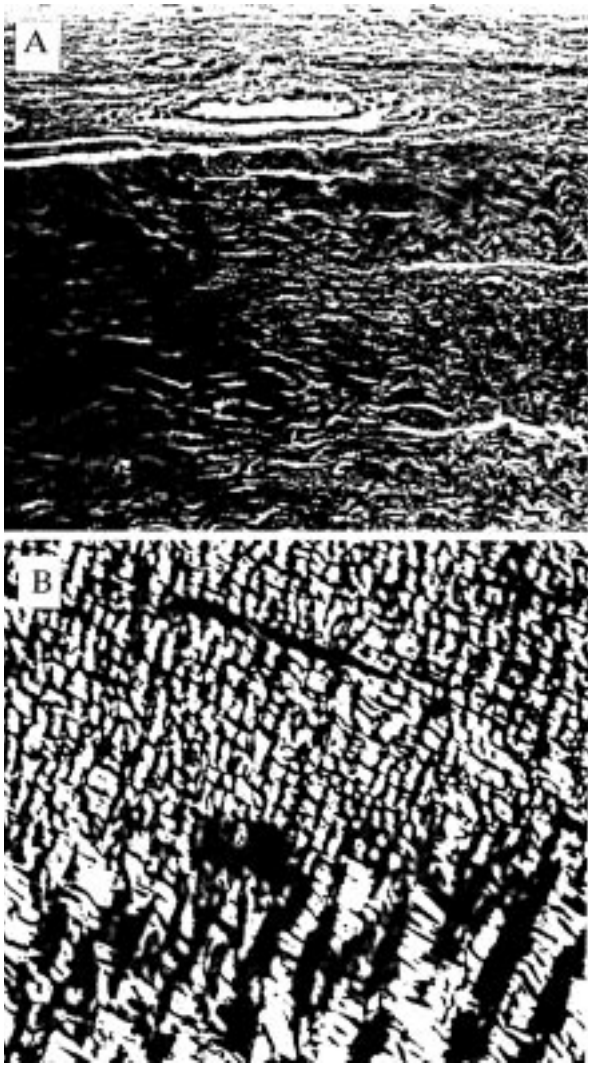

Figure 10 Optical microscopy of a cross-section of regenerated tissue after 30 days of operation and implantation of the polyacrylic hydrogel derived from vitamin E. (a) Staining with Masson's trichromic (100×); group without mobilization. (b) Staining with red sirius observed under polarized light (100×); group with free mobilization.

Acknowledgments

Financial support from CICYT, Mat 96-0981, and CAM, 08.4/0007/1997, are gratefully acknowledged.

References

1. L. PARKER, *Med. Biol.* **62** (1984) 105.
2. A. T. DIPLOCK, in ''Advances in pharmacology and therapeutics.'' Vol 8, edited by G. Olive (Pergamon Press, Oxford, 1979) p. 25.
3. F. R. COCHRAN, J. SELPH and P. SHUMAN, *Med. Res. Rev.* **16** (1996) 547.
4. J. M. ANDERSON, in ''Biomaterials science. An introduction to materials in medicine'', edited by B. D. Ratner, A. S. Hoffman, F. J. Schoen and J. E. Lemons (Academic Press, San Diego, California, 1996) p. 165.
5. W. E. M. LANDS, in ''Advances in drugs research'', Vol 14, edited by B. Testa (Academic Press, London, 1985) p. 147.
6. J. M. ARGILES, N. CARBÓ, P. COSTELLI and F. J. LÓPEZ-SORIANO, *Inc. Med. Res. Rev.* **18** (1998) 139.
7. I. FRIDOVICH, in ''Free radicals in biology'', Vol I, edited by W. A. Pryor (Academic Press, New York, 1976) p. 239.
8. S. SATO and T. TAKISHIMA, in ''Advances in pharmacology and therapeutics.'' Vol. 8, edited by G. Olive (Pergamon Press, Oxford, UK, 1979) p. 15.
9. M. A. SHUBERT, M. J. WIGGINS, K. M. DEFIFE, A. HILLTRER and J. M. ANDERSON, *J. Biomed. Mater. Res.* **32** (1996) 493.
10. C. ORTIZ, B. VÁZQUEZ and J. SAN ROMÁN, *Polymer* **39** (1998) 4107.
11. R. J. FORT and T. M. POLYZOIDIS, *Eur. Polym. J.* **12** (1976) 685.
12. D. K. OWENS, *J. Appl. Polym. Sci.* **13** (1969) 1744.
13. J. CRANK, ''The mathematics of diffusion,'' (Clarendon Press, Oxford, 1978) p. 239.
14. B. VÁZQUEZ, J. SAN ROMÁN, C. PENICHE and M. E. COHEN, *Macromolecules* **30** (1997) 8440.
15. D. A. LUSARDI and J. E. JR CAIN, *J. Foot–Ankle Surg.* **33** (1994) 443.
16. S. A. MEYERS, A. V. SEABER, R. R. GLISSON and J. A. NUNLEY, *J. Orthop. Res.* **7** (1988) 683.
17. S. BHATIA, P. BERGETHON, S. BLEASE, T. KEMPER, A. ROSIELLO, G. P. ZIMBARDI, C. FRANZBUN and E.L. SPATZ *J. NEUROSURG* **83** (1995) 897.

Received 7 February
and accepted 9 April 1999