PLGA bone plates reinforced with crosslinked PPF

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In this study a matrix of poly(propylene fumarate) (PPF) was crosslinked with N-vinylpyrrolidone (NVP), 2-hydroxyethylmethacrylate (HEMA), or a mixture of NVP and ethyleneglycol dimethacrylate (EGDMA) in the presence of poly(lactide-co-glycolide) (PLGA) to reinforce and preserve the form of PLGA bone plates. The degree of crosslinkage varied depending on the crosslinker as shown by the rapid and almost complete leaching of NVP upon incubation in phosphate buffered saline at 37 ° C in 900 h and retention of 92% of HEMA. With the reinforced bone plates extracted for 72 h at room temperature methylene chloride, the extracted PLGA from NVP/PPF, NVP-EGDMA/PPF, and HEMA/PPF were 75.42% (w/w), 59.52% (w/w), and 30.86% (w/w), respectively. The flexural modulus and compressive strength of the crosslinked PPF reinforced bone plates were higher than that of the unreinforced bone plate. Atomic force microscopy showed that NVP/PPF reinforced PLGA bone plates eroded substantially (a mean surface roughness of 19.319 nm) whereas NVP-EGDMA-PPF reinforced bone plate showed a distinct crystalline organization (and a higher roughness, 43.525 nm). In conclusion, we propose the consideration of NVP-EGDMA/PPF reinforced PLGA as a biodegradable orthopedic implant material that has a lower likelihood of warping or failing catastrophically than the currently available materials.

1. Introduction

Internal fixation devices fabricated from biodegradable polymers such as poly(lactide-co-glycolide) have several advantages over metallic devices: they do not corrode; they can be constructed with moduli closer to that of normal bone than metal devices and thus, as a corollary, avoid stress shielding; and finally, resorbability obviates the need of a second surgical procedure for removal of the device. However, their mechanical properties are no match to those of the metals and alloys, and unless strengthened they can not be used in many orthopedic applications including fracture fixation. Another disadvantage that is associated with biodegradable polymers is that they do not maintain their form after introduction into the body and undergo warping which is a primary cause for screw loosening in internal fixation devices. For example, when fractures created in the mandibles of six dogs were fixed with biodegradable PLA plates and screws, the devices could not be palpated at 24 weeks, suggesting a high degree of resorption [1]. Hollinger and Battistone [2] report that the PLA plates warped as they biodegraded, causing the retaining screws, also fabricated from PLA, to pull loose from the bone as shown in the paper by Weiler *et al*. [3]. In addition to warping, deformation and hollowing, implants showed focal areas of progressive degradation. This was observed particularly at screw holes and at osteotomy sites. Stahelin and Weiler [4] and Stahelin *et al*. [5] have reported clinical failure of PLGA-interference screws used for anterior cruciate ligament reconstruction due to hollowing of the screw.

We believe, to insure dimensional stability during degradation and to match the modulus and strength to that of bone, it is necessary to reinforce orthopedic biodegradable materials. The development of composites of metal and polymer plates (hybrid plates) was a step in this direction. Ferguson *et al*. [6] have designed a hybrid bone plate system that combines the torsional and bending rigidity of a metal plate with the axial compliance of a polymer insert. Biodegradable inserts further enhanced the performance of the new plate design, transferring less of the axial load to the plate as the inserts broke down.

In other attempts plates were reinforced by incorpora-

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tion of various materials. Parsons *et al*. [7] incorporated high modulus carbon fibers into PLA, but this approach compromised the biodegradability of the device. Skirving *et al*. [8] explored a completely non-degradable carbon fiber reinforced plate prepared from an epoxy cement. However, this did not solve the problem of warping. Some plates displayed permanent shape distortion acquired *in vivo*.

The fully biodegradable fixture is the ultimate choice and therefore strengthening the polymeric structure with other biodegradable materials or the same materials processed differently is preferred.

Reinforced and completely biodegradable plates were prepared by Christel *et al*. [9] who imbedded fibers of poly(glycolide) (PGA) in PLA plates. Since then considerable interest has focused on the concept of self-reinforced (SR) internal fixation devices. Thomson *et al*. [10] also showed that degradable hydroxyapatite fibers could increase the strength of a biomaterial.

Peltoniemi *et al*. [11] used titanium miniplates and biodegradable self-reinforced poly(L-lactide) (SR-PLLA) plates and assessed the consolidation of experimental craniotomy. The ''self-reinforcement'' was achieved by introducing PLLA fibers oriented parallel to the axis of the polymeric bulk of the plates. The biodegradable SR-PLLA plates promoted better osteotomy healing in the dynamically growing skull of young sheep than the titanium plates.

Peltoniemi *et al*. [12] later demonstrated that all SR-PLLA-plated osteotomy lines healed completely by 20 weeks, whereas none of the titanium plated lines had consolidated during a follow-up of one year.

In another study, mandibular unilateral body osteotomies were fixed with biodegradable self-reinforced poly(L-lactide) (SR-PLLA) multi-layer plates and screws in nine sheep [13]. The results showed that the SR-PLLA plates and screws were strong enough to fix the osteotomy and that the osteotomies healed mainly with callus formation.

Another method with which materials can be reinforced is by creating an inner, crosslinked structure to serve as a scaffold. This can be achieved by creating two meshes of intertwined polymer chains. Similarly, polymer chains of a specific type can be entrapped in the network of another. This semi-interpenetrating network has been shown to improve the mechanical properties of the polymers substantially [14].

The approach taken in this study is to reinforce poly(lactide-co-glycolide) (PLGA) by introducing in it a matrix of poly(propylene fumarate) crosslinked with a hydrophilic vinyl monomer. Poly(propylene fumarate) is a biocompatible and biodegradable, unsaturated polyester (Fig. 1). Thus, the crosslinked network would still be hydrolyzed because the crosslinks of the network would terminate at hydrolytically labile fumarate ester bonds. PLGA is also a polyester and biodegradable. Since the crosslinker is either a biocompatible, water soluble polymer (PVP and PHEMA), or a low molecular weight (therefore, easily excreted) chemical (EGDMA) it would not pose a risk. Here, the use NVP or HEMA as crosslinker might not seem appropriate because each is a monomer that can polymerize to high molecular weight chains. However, both because NVP was referred to as a crosslinker in a number of manuscripts [15–17] and also because NVP as well as another monomer, methyl methacrylate (MMA) were earlier shown to be permanently linked to PPF to form matrices, in this study, we will call NVP and HEMA crosslinkers. The main design of the scaffold material was earlier reported by Mikos and colleagues [15, 16] where PPF, an unsaturated condensation polymer, was crosslinked with NVP with the aim of developing a degradable, temporary replacement for trabecular bone. They, however, noted that unless reinforced by tricalcium phosphate the samples were very weak for bone replacement.

It is hypothesized that the crosslinked PPF-reinforced PLGA structure will have improved mechanical properties as bone plates (strength and modulus in compression,

a. The main material: Poly(lactic-co-glycolic acid) (PLGA):
$HO[OC-CH_2-O-CO-CH(CH_3)-O]_nH$

b. The main network material: Polypropylene fumarate (PPF):
$HO[CO-CH=CH-CO-O-CH(CH_3)-CH_2-O]_n-H$

c. The crosslinkers of PPF:
c1. Ethleneglycol dimethacrylate (EGDMA): $CH_2=C(CH_3)-COO-CH_2-CH_2-OOC-(CH_3)C=CH_2$

c2. 2-Hydroxyethyl methacrylate (HEMA): $CH_2=C(CH_3)-COOCH_2OH$

c3. N-Vinylpyrrolidone (NVP):

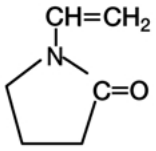
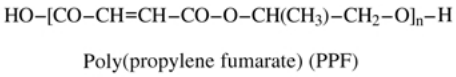
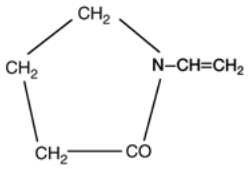


Figure 1 Formulas of the components of the molecular reinforcement system.



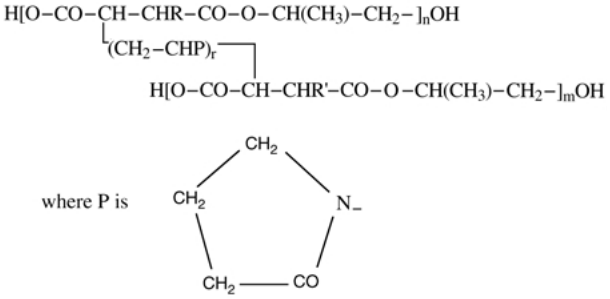
Poly(propylene fumarate) (PPF)

(a)



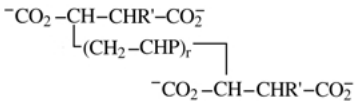
N-Vinyl-2-pyrrolidone

(b)



Polypropylene fumarate crosslinked with N-vinyl-2-pyrrolidone

(c)



soluble products of hydrolysis of crosslinked PPF

(d)

Figure 2 Schematic presentation of formation of crosslinked PPF structure and its degradation products [from Ref 17].

tension, and bending) in comparison to unreinforced PLGA. The support provided by the matrix to the main structural element, PLGA, is expected to protect the device against dimensional instability such as warping or hollowing that arises from unequal rates of degradation or autocatalytic degradation observed with polyesters.

When this matrix was created in the presence of PLGA the changes in the mechanical properties were not very encouraging. Although a high degree of the crosslinking could be achieved (90% of the PPF was crosslinked [17]), the resultant materials could not maintain their mechanical properties upon *in vitro* and *in vivo* testing [17–19]. A similar result was obtained with a polyethylene glycol-PPF copolymer [20]. Hypothesizing that the cause of the inadequate *in vitro* and *in vivo* behavior was the presence and the length of the hydrophilic crosslinker, ethyleneglycol dimethacrylate (EGDMA) was tested as a short and relatively less hydrophilic crosslinker, and 2-hydroxyethyl methacrylate (HEMA) was tested as a less hydrophilic monomer to replace NVP.

An important point at this stage is to show that the crosslinkers introduced improve the mechanical properties and form retention. These are achieved readily by mechanical testing and *in vitro* incubation. The proof of presence of a matrix within the bone plates is another matter. Indirect evidence is gathered by analysis of degradation products, and change of physical and mechanical properties. Another, a relatively more direct approach is to examine the bone plates with atomic force microscopy (AFM), a method increasingly being used to study and quantify surface properties of materials. It was chosen to observe the implants in their untreated, natural form unlike scanning electron microscopy where the sample is completely dried and coated with a conductor (e.g. gold). AFM is a novel, versatile tool in the investigation of material structures. It is essentially a scanner that creates topographical maps of surfaces. A very sharp tip is located at the free end of a cantilever and follows the contours of the surface as it is moved over it. The deflections of the tip are measured by an optical detector and recorded by a computer. The data can be used to create a 2D or 3D image and measure surface roughness. It started becoming very important in the life sciences because most of the structures are highly hydrated and fragile. Its main importance lies in the ability to visualize these hydrated materials in intact form without any treatment as in scanning electron microscopy SEM where the sample needs to be dried and coated with conducting materials that dramatically alter biological and other delicate and/or highly water containing samples [21–26]. With AFM, however, it was possible to examine very fragile hydrogel materials like soft contact lenses in their swollen form and measure their surface roughness [24]. The method does not just enable the researchers to study the soft, hydrophilic synthetic or biological specimens. It also allows their surface topography to be studied in detail and helps reveal structures and organization. In a study with AFM, the surface of biodegradable blends of poly(sebacic acid anhydride) (PSA) and PLA were examined [27]. They were able to follow the degradation of the components in real time and record the phase separations and preferential degradation of the PSA component leaving behind surfaces enriched in PLA. It was thus possible to expose the PLA morphology. With the use of a similar technique, scanning force microscopy, close packed, needle like organization of crystals of poly(butene-1) were shown with a resolution of the order of nanometers [28]. The authors claimed to show individual poly(butene-1) molecules. In another study, lamellar organization of polyethylene [29], and various spherulitic surfaces [30, 31] could be visualized. We, therefore, thought that it would be possible to employ this methodology to show differences in the surface topographies of PLGA bone plates with and without reinforcement with crosslinked PPF, and also to detect changes upon degradation.

In addition to examination with other physical and mechanical methods, the bone plates prepared in this study were, therefore, examined with AFM for the

presence signs of specific orientations on their surfaces and changes in them upon incubation in distilled water for 48 h were studied to gain information on the influence of the presence of matrices.

2. Materials and methods

2.1. Materials

PLGA-85: 15 was purchased from Boehringer-Ingelheim (Resomer 858, Mw ~ 232,000 daltons) and purified by precipitation from an acetone solution (50 mg/ml) into 2-propanol (isopropyl alcohol).

Poly(propylene fumarate) (PPF, Mw ~ 4500 daltons with GPC, heterogeneity index 2.10) was synthesized from equimolar fumaric acid and propylene glycol in the presence of p-toluene sulfonic acid (0.45%, w/w) catalyst and n-butyl hydroquinone (0.3%, w/w) inhibitor according to Wise *et al*. [32]. N-vinylpyrrolidone (NVP) was purchased from Aldrich (USA) and used after vacuum distillation. Benzoyl peroxide (BP) (Aldrich), methylene chloride (Fisher Scientific) and glacial acetic acid (Fisher Scientific) were used as received.

PPF was crosslinked with NVP, EGDMA or HEMA using benzoyl peroxide (Aldrich Chemical Co.) as the initiator. Each plate type consists of (as weight %) PLGA 85:15 (62.5%), PPF (20.8%), and benzoyl peroxide (2.1%). In addition, NVP/PPF had NVP (14.6%), NVP-EGDMA/PPF had NVP (8.3%) and EGDMA (6.3%), and HEMA/PPF had HEMA (14.6%) as crosslinker. The implant names were designed to reflect their interpenetrating nature (e.g. HEMA/PPF) and to avoid confusion with the compound introduced as the crosslinker (HEMA).

2.2. Plate preparation

PPF (1.0 g) and PLGA (3.0 g) were dissolved in approximately 5 ml of acetone. Then, benzoyl peroxide (0.1 g) and the crosslinking agent(s) were added, and mixed to form a paste. This mixture was maintained in a lyophilizer at room temperature for approximately 5 min or until the presence of acetone could not be detected by odor. The mixture was then placed in a ceramic mold, heated in an oven at 70 °C overnight, and then at 100 °C for 1 h for a final cure. Afterwards the polymer was introduced into a 2.54 cm diameter steel mold and compressed at 2 ton pressure and 65 °C for 1 h.

2.3. *In vitro* release of leachables

In vitro tests to determine the amount of leachables released from the three formulations and the control were performed on cylindrical disks (0.635 cm diameter and *ca*. 3 mm thickness) ($n = 4$). They were placed in 50 ml vial with 10 ml of phosphate-buffered saline solution (PBS, 0.1 M, pH 7.40), in a water bath at 37.0 °C agitated at 60 cycles/min. The solutions were analyzed over time with a Cary UV Spectrophotometer. Samples were monitored at 202 nm and 232 nm for the quantification of NVP (monomer) and PVP (its polymer), and 208 nm for HEMA and its polymer, pHEMA. After each measurement, the samples were returned to their vials and 10 ml of fresh PBS was added.

2.4. Swelling tests

Samples treated as in the previous section were blotted, and wet weights were determined. After each measurement, the samples were returned to their vials and fresh PBS (10 ml) was added.

2.5. Mechanical properties

Samples (3.4 × 13 × 65 mm) were prepared and used in the three-point bending tests performed on an Instron (Model 8511) with a 500 lb load cell, span of 50 mm, ramp start of 30 mm, speed 0.1 mm s^{-1}, 10 pt s^{-1} and 3624 total point conditions.

Compression testing was carried out with the same Instron with 2500 lb load cell (Serial No. 75084), ramp 4 mm, 20 pt s^{-1} data sampling conditions. Compression and three-point bending tests were repeated on samples ($n = 4$) that had been incubated *in vitro* at 37 °C for 120 h in PBS and rinsed with distilled water, blotted, and kept moist until testing. A modified Labview program (National Instruments, USA) was used to run the tests and analyze the results.

2.6. Extraction of PLGA in the bone plates with methylene chloride

The objective of this test was to obtain evidence about the existence of the reinforced structure consisting of crosslinked PPF distributed in PLGA by dissolving out the PLGA and other non-polymerized components of the reinforced bone plate. To achieve this, samples ($n = 3$) were incubated in methylene chloride (4 ml) at room temperature for 24 h. The samples were then centrifuged, methylene chloride was pipetted off, and replaced with 6 ml fresh methylene chloride. Extraction was repeated at the end of 48 h. At the end of three days, solvent was removed, and the samples were lyophilized and weighed. This yielded the extractable fraction (%, w/w), the remainder being the crosslinked PPF network and the inextractable PLGA.

2.7. Atomic force microscopy (AFM)

Samples (3 × 3 × 1 mm) of NVP/PPF, NVP-EGDMA/PPF and HEMA/PPF were cut from the bone plates and attached to AFM specimen disks (15 mm diameter, Ted Pella Inc., Redding, CA, USA.). They were then incubated in distilled water at room temperature for two days to initiate the removal of leachables. These samples were examined alongside untreated control of each formulation in a Nanoscope III AFM (Digital Instruments, Santa Barbara, CA), in contact mode, in air, using square pyramidal tip of a Si_3N_4 cantilever. The atomic force microscope is a topographical tool that results in a 3D image and can be used in quantitative measurements such as surface roughness. The surface roughness analysis was carried out using the available DI software for the AFM, and mean surface roughness (R_a) and maximum height (R_{max}) were calculated. These are defined in the Digital Instruments' Nanoscope III Control System User's Manual version 3.0 as:

R_a: average deviation from the center plane, and

R_{max}: the difference between the highest and lowest z-position relative to the center line, respectively.

3. Results and discussion

The aim of this investigation was to prepare reinforced PLGA plates using crosslinked PPF as a reinforcing structure, to characterize these novel bone plates mechanically, physically, and morphologically, and show evidence of the existence of the reinforcing structure.

3.1. Extraction with methylene chloride

The objective of this test was to reveal the molecular reinforcing structure, the crosslinked PPF by dissolving out the PLGA and any other non-polymerized components in methylene chloride. The extractables are nonpolar chemicals chemically not bonded to the matrix (e.g. PLGA) and any lightly crosslinked or uncrosslinked PPF. It was expected that when the PPF is heavily crosslinked a fraction of the PLGA chains would not be able to disentangle and leave the reinforced plate upon extraction. The size of this fraction would depend on the extent of reinforcement, and the crosslinker length and hydrophilicity.

After the first 24 h in the extraction medium, gross macroscopical observation revealed that:

1. NVP/PPF reinforced bone plate crumbled into irregular shaped particles.
2. HEMA/PPF reinforced bone plate was substantially swollen but maintained its form and integrity.
3. NVP-EGDMA/PPF reinforced bone plate was able to maintain its form and integrity, and did not swell as much as the HEMA reinforced bone plate.

Sample appearances did not change significantly between 24 and 72 h.

The weight decreases upon extraction with methylene chloride were $74.42 \pm 10.96\%$ (w/w), $59.52 \pm 13.70\%$ (w/w), $30.86 \pm 6.95\%$ (w/w), for NVP/PPF, NVP-EGDMA/PPF, and HEMA/PPF reinforced bone plates, respectively. The largest component of the bone plates, PLGA, constitutes 62.5% (w/w) of an untreated bone plate. The NVP/PPF reinforced plate weight decrease (*ca*. 75%) implies that in addition to all the PLGA some other components were also extracted. In contrast, NVP-EGDMA/PPF reinforced plate appears to retain some of its PLGA content and all its matrix components. HEMA/PPF reinforced bone plate also entrapped a significant amount of PLGA that it did not release. These results indicate that when used alone NVP is the least suitable crosslinker for constructing a reinforced bone fixture.

3.2. *In vitro* leaching of bone plate crosslinkers

N-vinylpyrrolidone leached out of NVP/PPF and NVP-EGDMA/PPF formulations was detected at two wavelengths; 202 nm for the polymer, PVP, and 232 nm for the monomer, NVP. With HEMA/PPF reinforced bone plates, leaching was detected at a single wavelength of 207 nm corresponding to monomeric HEMA. EGDMA was not studied because it is insoluble in water so it does not have an absorbance in water.

The amounts of N-vinylpyrrolidone and HEMA leached into the solution are presented in Table I.

NVP/PPF reinforced bone plate, containing only NVP as a crosslinker, had the most rapid and the largest degree of release of combined PVP and NVP (58% in 100 h and 93% in 900 h).

NVP-EGDMA/PPF reinforced bone plate contained half the NVP of NVP/PPF reinforced bone in its preparation formulation and it did not lose NVP + PVP at levels comparable to NVP/PPF. This bone plate lost 42% of its NVP in 900 h in comparison to 93% by NVP/PPF reinforced bone plate. This indicates that NVP of NVP-EGDMA/PPF has become more highly incorporated into the bone plate reinforcing matrix.

HEMA/PPF reinforced PLGA bone plates retained the highest proportion of the crosslinking agent. Its loss was less than 10% (w/w) in 900 h. This obviously is a substantial improvement over NVP/PPF reinforced bone plates.

3.3. Swelling test

During the 174 h test duration PLGA did not swell appreciably (3.1%) because it is highly hydrophobic (Table II). Among the samples tested, NVP/PPF reinforced bone plates had the highest rate of solvent absorption and, therefore, increase in weight (18.5% in one day, 25.3% in 72 h) (Table II). This absorbed water was all on account of NVP and its polymer, PVP, because as was shown with NVP/PPF, PLGA does not absorb an appreciable amount of water. After 72 h, the NVP/PPF reinforced plate started to lose weight. Since 72 h is too early for an extensive degree of degradation (at least 15 days is needed [33]) it can be assumed that upon rapid swelling, initially the remaining unreacted monomer (NVP) would leach out of the plate. Then its polymer, poly(N-vinylpyrrolidone), would leave the structure if not involved in the crosslinkage process and stayed linear. This explanation agrees with the substantial leaching of NVP and PVP from the NVP/PPF reinforced sample (Table I). A similar loss of crosslinker from PPF crosslinked with PEG was reported with a much earlier onset time (one day) and to a much higher degree (40–60% of the weight) proving that crosslinking of PPF is not that extensively achieved [19, 20].

HEMA/PPF and NVP-EGDMA/PPF were both slower than NVP/PPF in absorbing water but they continued

TABLE I *In vitro* leaching of the crosslinked from crosslinked PPF reinforced bone plates (in PBS at 37 °C in shaking water bath) ($n = 4$)

Sample	Crosslinker leached in time (%, w/w)	
	100 h	900 h
NVP/PPF	58	93
NVP-EGDMA/PPF	8	42
HEMA/PPF	1	8

TABLE II *In vitro* swelling of the crosslinked PPF reinforced and unreinforced bone plates (in PBS at 37 °C in shaking waterbath) ($n = 4$)

t (h)	Swelling (%, weight water/weight dry plate)			
	PLGA	NVP/PPF	NVP-EGDMA/PPF	HEMA/PPF
24	1.5 ± 0.4	18.5 ± 3.1	10.3 ± 1.0	10.6 ± 2.0
53	1.8 ± 0.4	23.9 ± 2.0	16.5 ± 1.3	15.6 ± 2.7
72	2.3 ± 0.6	25.3 ± 2.8	19.5 ± 1.8	18.9 ± 2.8
174	3.1 ± 0.8	22.4 ± 0.8	31.4 ± 3.6	31.9 ± 3.8

absorbing water throughout the test duration. In the end, these two had the highest degrees of swelling.

In a typical hydrogel, normally, water absorption is complete within 2–3 days [34, 35]. In this study the lower rate of absorption and equilibration is most probably due to the presence of the hydrophobic component, PLGA, in the structure.

3.4. Mechanical testing

Flexural and compressive strengths, and flexural modulus data of the bone plates ($n = 4$) incubated in PBS for 120 h at 37 °C and the untreated bone plates are presented in Table III.

In all tests, the mechanical properties of dry, untreated NVP/PPF, NVP-EGDMA/PPF, and HEMA/PPF samples showed that they have equal or greater load carrying capabilities than the control, unreinforced PLGA, unlike earlier results with similar formulations [36], where, in dry state, the mechanical properties of the reinforced plates were inferior in both untreated and treated form. The values obtained in this study are also distinctly higher than those of TCP-loaded NVP/PPF structures designed for use as a trabecular bone substitute [15, 16].

Flexural strength shows that NVP has a negative contribution to the properties of PLGA while EGDMA and HEMA incorporation compensates for this, as observed in the data of the treated samples (Table III). Since NVP-EGDMA/PPF formulations contain NVP as co-crosslinker, the improvement in the properties can be attributed to the partial replacement of NVP implying that a complete replacement would lead to a better result.

The positive influence of the reinforcement is more apparent in flexural modulus and compressive strength, both in untreated and treated form. HEMA/PPF has the highest value among both the treated and untreated samples, followed by NVP-EGDMA/PPF, NVP/PPF and unreinforced PLGA. The samples with the highest compressive strengths are NVP/PPF and HEMA/PPF among the untreated and treated samples, respectively. In all cases unreinforced PLGA has the lowest values.

Incubation in PBS decreased the mechanical properties of all the bone plates by *ca*. 30–50%. It is apparent that the plates absorbed water mostly due to their N-vinylpyrrolidone or HEMA contents, because PLGA water absorption is very low (Table II). Since a significant amount of material is not leached in such a short time (two days), the loss of mechanical properties is most probably due to the plasticization effect of water. A similar trend was earlier observed with various NVP/PPF reinforced PLGA [18]. A more drastic reduction in tensile strength than observed here was reported with poly(L-lactide) within 1 h of contact with distilled water and this supports the explanation *via* plasticization [36]. Suggs *et al*. [20] reported a significant mechanical property loss (down to 20% of its original value) in the first three weeks of incubation in PBS with their NVP crosslinked poly(propylene fumarate-co-polyethylene glycol) (NVP/PPF-co-PEG) copolymer. A controversial report is that of Yaszemski *et al*. (1996) [15] whose TCP loaded NVP crosslinked PPF samples showed increased mechanical properties upon incubation in PBS at 37 °C. Although an explanation was not offered, one possibility is that degradation and leaching out of NVP/PPF matrix leaves behind a product enriched in TCP and this would lead to increase in mechanical properties.

In conclusion, it is clear that the reinforcement improves the mechanical properties (especially observed in flexural modulus and compressive strength) and this improvement is higher if the crosslinker is less hydrophilic or shorter than NVP.

3.5. Atomic force microscopy

3.5.1. Surface topography

The atomic force micrographs of treated (incubated in distilled water (DW) for 48 h) and untreated samples of NVP-EGDMA/PPF, HEMA/PPF, and NVP/PPF reinforced bone plate surfaces are presented in Fig. 3. It is observed that the uppermost 100 nm of the surface of the NVP/PPF formulation (Fig. 3b3) has eroded uniformly without any surface topographical features. The NVP-

TABLE III Mechanical properties of treated and untreated crosslinked PPF reinforced bone plates

Sample	Flexural strength (MPa)	Flexural modulus (GPa)	Compressive strength (MPa)
PLGA: Untreated	61.10	2.74	56.24
Treated	25.75	1.49	14.61
NVP/PPF: Untreated	41.57	2.78	73.77
Treated	19.66	1.15	29.13
NVP-EGDMA/PPF: Untreated	47.00	3.18	56.80
Treated	24.30	1.69	29.40
HEMA/PPF: Untreated	63.70	4.12	60.21
Treated	32.03	1.82	38.38

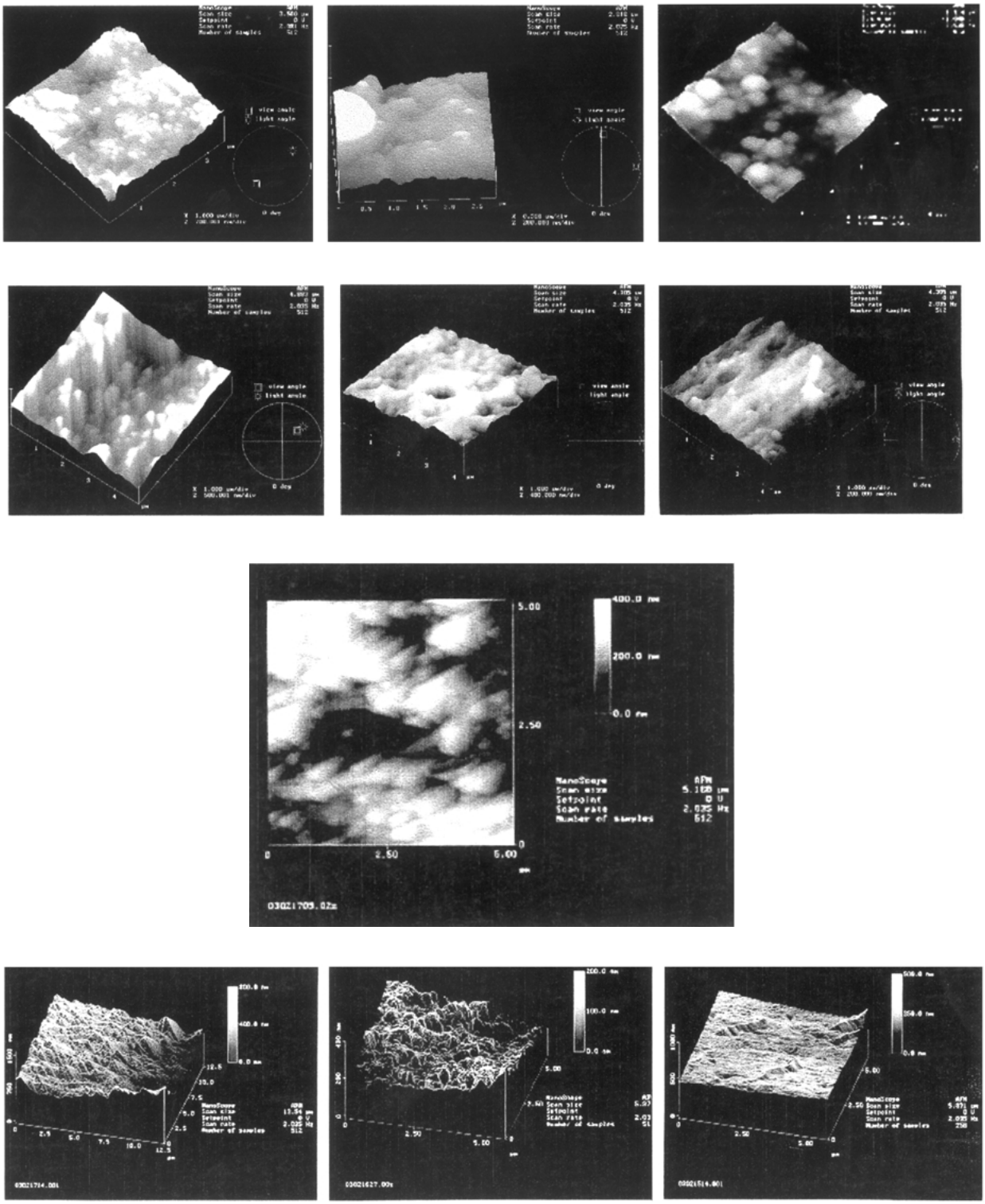


Figure 3 Atomic force micrographs of NVP-EGDMA/PPF, HEMA/PPF, and NVP/PPF formulations before and after *in vitro* treatment (incubation in distilled water at 37 °C for 48 h in a shaking waterbath). a: untreated (surface) (a1: NVP-EGDMA/PPF, a2: HEMA/PPF, a3: NVP/PPF); b: *in vitro* treated (surface) (b1: NVP-EGDMA/PPF, b2: HEMA/PPF, b3: NVP/PPF); c: *in vitro* treated surface of NVP-EGDMA/PPF (view from another angle); d: *in vitro* treated (line micrographs) (d1: NVP-EGDMA/PPF, d2: HEMA/PPF, d3: NVP/PPF).

EGDMA/PPF and HEMA/PPF samples (Figs 3b1 and 3b2), on the other hand, eroded creating distinct surface features. Especially, the NVP-EGDMA/PPF reinforced bone plates exhibited a regular hexagonal crystals oriented diagonally in the micrograph (Fig. 3c).

The same data was processed to obtain line plots (Figs 3d1–3) and it revealed a very smooth surface for NVP/PPF, and a granular surface with almost spherical particles (*ca*. 350 nm in diameter) with HEMA/PPF. With NVP-EGDMA/PPF a striking surface composed of rectangular prisms with a thickness of *ca*. 200 nm is obtained. These imply that the uppermost regions of the surfaces of the NVP/PPF samples eroded evenly while the others, although eroded to some extent, still maintained an underlying structure that resisted significant erosion. Even though the bone plates were homogeneous in composition material loss can not be expected to occur evenly because the crystallinity of components are different and would have different rates of water ingression. When a component is more

TABLE IV Changes in surface roughness and maximum heights upon *in vitro* treatment (48 h at room temperature, in distilled water)

Sample	Mean surface roughness (R_a), nm		Maximum height (R_{max}), nm	
	Untreated	Treated	Untreated	Treated
NVP/PPF	34.464	19.319	305.81	192.71
NVP-EGDMA/PPF	57.690	43.525	459.79	374.98
HEMA/PPF	35.761	29.958	306.03	294.53

amorphous, then that component absorbs solvent more rapidly, it can swell or be eroded away leaving behind the more crystalline one. This leads to a surface enriched in the more crystalline component. A similar phenomenon was reported by Davies *et al*. [27] who with poly(lactic acid) (PLA) and poly(sebacic acid) (PSA) blends observed enrichment of the surface in PLA because of PSA was more amorphous, absorbed more solvent and, therefore, degraded more rapidly.

3.5.2. Surface roughness

The surface roughness of NVP/PPF, NVP-EGDMA/PPF and HEMA/PPF bone plates were analyzed using the software available for the AFM (Table IV).

The mean roughness values (R_a) for the untreated samples are 36.464 nm, 57.690 nm, and 35.761 nm for NVP/PPF, NVP-EGDMA/PPF, and HEMA/PPF, respectively. Upon incubation in distilled water for two days the roughness values became 19.319 nm, 43.525 nm and 29.958 nm. Based on the above data, the roughness order after treatment can be presented as: NVP-EGDMA/PPF > HEMA/PPF > NVP/PPF. When the maximum height (R_{max}) values of these samples, obtained through the same measurement series, are compared it is observed that this order is maintained (Table IV). The interesting thing is that upon treatment, the roughness order is still the same and the largest decrease in the values is observed with NVP/PPF. These can be interpreted as erosion being the most effective on the least crosslinked sample (NVP/PPF) and is less effective on the more reinforced samples (NVP-EGDMA/PPF and HEMA/PPF).

4. Conclusions

Biodegradable internal fixation devices fabricated from polymers such as PLA or PLGA are advantageous over metallic devices because they do not corrode; they do not lead to stress shielding and the need of a second surgical procedure for implant removal is avoided. They need to be strengthened so that their mechanical properties could approximate those of compact bone. Otherwise, they can not be used in many orthopedic applications including fracture fixation. Also, biodegradable polymers can not maintain their form after introduction into the body and undergo ''warping'' leading to screw loosening and implant failure in internal fixation devices. The approach taken in this study was to apply a reinforcement approach as a means of strengthening bone plates. As the major structural element poly(lactide-co-glycolide) 85 : 15 was selected and it was reinforced by a scaffold of poly(propylene fumarate) crosslinked with a vinyl monomer. We have hypothesized that this reinforced composite would have improved structural integrity in comparison to unreinforced PLGA.

The data obtained during extraction with methylene chloride and *in vitro* leaching reveal that NVP/PPF does not have an extensive organization in its structure. Both the non-polar solvent-soluble compounds, PLGA and PPF, are easily extracted and the crosslinker, NVP (and may be its polymer PVP), almost completely leave the bulk in water. Both NVP and PVP are biocompatible but not biodegradable. Their loss *in vitro* is, therefore, caused by their dissolution and leaching. The NVP/PPF structure probably contains PLGA chains which are loosely and very sparsely entangled with very short range crosslinked PPF networks. The hydrophilic crosslink segments swell and cause rapid material loss. As a result, the removal of PLGA by methylene chloride is facile and extensive, as is the leaching of NVP or PVP. This is why a featureless topography is obtained in the AFM work.

At the other extreme is NVP-EGDMA/PPF where the crosslinked PPF matrix is probably more extensive, entangling the PLGA chains more effectively. In addition, EGDMA, due to its being a crosslinker but not a monomer could not form long chains as does NVP, and also due to its hydrophobicity, does not lead to swelling in aqueous media, thus further slowing erosion. The fibrous, close-packed structures observed in the AFM are possibly a result of an extensive crosslinked matrix formation leading to preservation of form, and resistance to dissolve completely in water.

Finally, AFM proved its worth as a non-destructive method of surface analysis in examining hydrophilic surfaces without any treatment.

We believe that the implants constructed of NVP-EGDMA/PPF with lesser NVP than used in this study will probably be of significant value in fracture fixation applications.

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