Colloid Polym Sci (2008) 286:357–363
DOI 10.1007/s00396-007-1793-7

SHORT COMMUNICATION

Synthesis of biocompatible nanocomposite hydrogels as a local drug delivery system

Jozsef Bako · Marta Szepesi · Adrienn J. Veres · Csaba Cserhati · Zsuzsa M. Borbely · Csaba Hegedus · Janos Borbely

Received: 4 May 2007 / Revised: 23 October 2007 / Accepted: 25 October 2007 / Published online: 25 November 2007

Abstract Nanocomposite biocompatible hydrogels (NCHG) were synthesised as model systems for in situ cured potentially local drug delivery devices for curing periodontal infections. The composite consists of the following components: nanoparticles (NPs), matrix gel, and chlorhexidine (CHX) as antibacterial drug. The NPs were obtained by free radical initiated copolymerization of the monomers, 2-hydroxyethyl methacrylate (HEMA) and polyethyleneglycol dimethacrylate (PEGDMA), in aqueous solution. The same monomers were used to prepare crosslinked matrices by photopolymerization. NCHGs were obtained by mixing NPs, monomers, and drug in an aqueous solution then crosslinked by photopolymerization. Mechanical properties, swelling behavior, and the kinetics of drug release have been investigated. It was found that compression strength values increased with increasing ratio of the crosslinker PEGDMA. Incorporation of NPs into the matrix resulted similar compression strength as the matrix hydrogel. The hydrated NCHGs swelled more slowly but admitted more water. The drug was incorporated in NPs by swelling in CHX aqueous solution or added to the solution of monomer mixture followed by photopolymerization. Studies of release kinetics revealed that on average 60% of the loaded drug was released. The most rapid release was observed over a 24 h period for matrix gels with low crosslinking density. For NCHGs, the release period exceeded 48 h. An unexpected result was observed for NCHGs without drug in the NPs. In this case, increasing release was observed for the first 24 h. Thereafter, however, the apparent quantity of detectable drug decreased dramatically.

Keywords Nanocomposite · Hydrogel · Photopolymerization · Chlorhexidine diguconate · Local drug delivery

J. Bako · A. J. Veres · J. Borbely
Department of Colloid and Environmental Chemistry, University of Debrecen, Debrecen 4010, Hungary

M. Szepesi · C. Hegedus
Department of Prosthetic Dentistry, University of Debrecen, Debrecen 4010, Hungary

C. Cserhati
Department of Solid State Physics, University of Debrecen, Debrecen 4010, Hungary

Z. M. Borbely
Department of Food Hygiene, Szent Istvan University, Budapest 1078, Hungary

J. Borbely (✉)
BBS Nanotechnology, Debrecen 4225, Hungary
e-mail: jborbely@delfin.unideb.hu

Introduction

Hydrogels are three dimensional hydrophilic crosslinked polymer networks, which are capable of swelling in water. They have soft and elastic properties, and being highly hydrophilic there are similarities to natural tissues [1]. Their widespread application is well known, for example in the cosmetic industry as moisturizing creams and emollients. Hydrogels have important role in ophthalmology as contact lenses [2], or as a local drug delivery system to treat glaucoma [3]. In dermatology it is used for rehydrating necrotical crusts [4]. Hydrogels are also important in dentistry. For example, PerioChip is a hydrogel, used in periodontology as a drug delivery device for the release of CHX [5]. Because of its efficiency, allowing reduction in drug dosage among other advantages, the application of

controlled release systems is growing. Commonly, these involve matrices composed of biocompatible polymers [6–11].

Mono and multifunctional acrylates and their derivatives are commonly used monomers for synthesis of biocompatible hydrogels. The synthetic methods vary, for instance thermal- [12], oxidation-reduction- [13] or photopolymerization [14, 15]. The use of the photopolymerization is growing, and is replacing older methods [16–21].

Among the large number of monomers that are available, HEMA is well known and is commonly used as a crosslinker, providing a very good biocompatible system. The properties of gels based on this monomer were modified by applying other materials for example different crosslinkers, and recently, the use of nanoparticles is exponentially increasing [22–27]. Extensive use of the novel hydrogel nanocomposites is made in bone grafting or tissue regeneration studies [28, 29], and these gels are also synthesized and investigated, e.g., as drug formulation systems [30, 31].

In this paper, the preparation of nanocomposite hydrogels (NCHGs) is described. The aim of this work is to study the effect on release kinetics of a drug incorporated into such a nanoparticle-gel matrix system. In addition, the effect on release kinetics of nanoparticle-matrix gel porosity, which is a function of cross-linkers density, will also be determined.

Experimental part

Materials

2-Hydroxyethyl methacrylate (97%, from Sigma-Aldrich, Steinheim, Germany) was purchased as monomer, poly (ethylene glycol) dimethacrylate (Mn:550, from Sigma-Aldrich, St. Louis, MO) as crosslinker, and anthraquinone-2-sulfonic sodium salt (~99%, Fluka AG. Buchs SG) as photoinitiator was applied. Chlorhexidine-di-gluconate, dental application grade (20% solution from Spektrum 3D, Hungary), was obtained as active substance. All materials were used as received without further purification.

Preparation of nanoparticle

HEMA and PEGDMA monomers were dispersed in deionized water (40 ml) containing sodium lauryl sulfate (SLS), an anionic surfactant. The overall concentration of monomers was 4 wt%, and the concentration of SLS was 2.4 wt%. The composition feed of monomers was 50 mol% of HEMA and 50 mol% of PEGDMA. The batch copolymerization was performed in a 150 ml three-necked, round-bottom flask. It was purged with nitrogen for 25 min on ambient temperature to remove the oxygen dissolved. Solution of potassium persulfate initiator (the concentration was 0.2 wt%) was added and free radical polymerization was performed at 60 °C. The reaction mixture was continuously stirred by magnetic stirrer under nitrogen atmosphere. The polymerization time was 2 h. The sample was cooled and dialyzed against water for a week and then was freeze-dried in a Virtis Freeze Drier (CHRIST ALPHA 1–2) under vacuum at −52 °C for 4 days.

Synthesis of hydrogels and nanocomposites (photopolymerization)

HEMA–PEGDMA hydrogels were prepared in aqueous media. The total concentration of the monomers was 30 wt%. Hydrogels were prepared using different feed mol ratios as 90:10, 75:25, 50:50, 25:75, and 10:90. In addition 1.0 mol% of photoinitiator calculated for monomers was measured and these "solutions" were homogenized by hand and then by ultrasonic bath. The NCHGs that were made by the same method in which the HEMA/PEGDMA feed was 50:50 and 15 wt% NPs was dispersed in the clear, yellowish aqueous "solution" of monomers and photoinitiator. The prepared NPs were loaded with CHX that they were swelled in small amount of drug solution for 48 h. Then, these loaded particles were freeze-dried. The solid, loaded particles were then dissolved in the aqueous "solution" of monomers and initiator in order to form the NCHGs.

The mixtures were poured into cylinderical molds made of polypropylene, with a diameter of 9 mm and the prepared sample height varied from 4 to 10 mm. The top of the holders were closed with cover slips to avoid the inhibition effect of the oxygen. The initiation of photopolymerization was performed by Kulzer Palatray CU lamp source supplying blue light with 420 nm wavelength and 1.50 W per cm^2 irradiation energy. The reaction time was adjusted for 25 min due to the larger thickness of the specimen. Four types of samples were prepared for release studies where the CHX was added to the reaction mixture before photopolymerization. In the case of matrix gels without NPs, CHX was dissolved in the aqueous solution of monomers and then crosslinked (Matrix gel). For NCHGs, the NPs were swelled in aqueous solution of CHX and then added to the mixture of monomers and initiator, and then crosslinked (NCHG 2, 3). The NCHG1 was prepared with NPs but drug was only in matrix. The composition of gel particles is summarized in Table 1.

Mechanical assay

Hydrogel specimens were investigated with INSTRON 4302 Mechanical Analyser. The compression tests were performed on cylindrical samples described above with the full scale load range at 0.1 kN, and the crosshead speed at

Table 1 Composition of nanocomposites and matrix gels prepared for release analysis

Sample number	HEMA (g)	PEGDMA (g)	Initiator (g)	NPs (g)	CHX 20%(ml)		H_2O (g)
					in matrix	in NPs	
Matrix gel	**0.1069**	**0.4930**	**0.0051**	**0**	**0.075**		**1.32**
NCHG1	**0.1069**	**0.4930**	**0.0051**	**0.3**	**0.075**	**0**	**1.02**
NCHG2	**0.1069**	**0.4930**	**0.0051**	**0.3**	**0**	**0.225**	**0.87**
NCHG3	**0.1069**	**0.4930**	**0.0051**	**0.3**	**0.075**	**0.225**	**0.795**

In the matrix gel (5:5—HEMA/PEGDMA), CHX was 15 mg; in NCHG1, the CHX was 15 mg only in the matrix; in NCHG2, CHX was 45 mg only in NPs; in NCHG3, the total amount of CHX was 60 mg divided in two parts—15 mg in the matrix and 45 mg in NPs were loaded.

2 mm/min. The cylindrical samples had a diameter of 9 mm and specimen length of 4 and 9 mm, for the matrix hydrogel and for NCHG systems, respectively.

Dynamic Light Scattering (DLS)

Hydrodynamic diameter (HD) of NPs was measured with a BI-200SM Brookhaven Research Laser Light Scattering goniometer equipped with a NdYAg solid state laser at an operating wavelength of λ_0=532 nm. Measurements of the average size of NPs were performed at 25 °C with an angle detection of 90° in optically homogeneous quartz cylinder cuvettes. The samples were prepared from the reaction mixture after dialysis, and from freeze-dried samples. The concentration of the dispersion of polymer was 100 μg/ml.

Scanning electron microscopy analysis

The hydrogels were dried at 110 °C for 2 h and sputter-coated with gold for 30 s The plasma current was 18–20 mA, while the sputtering Ar pressure was about 10–20 mPa during the coating. The thickness of the deposited Au layer was about 100–200 nm. Samples were imaged using scanning electron microscope (Hitachi S4300 CFE, Tokyo, Japan) at 1, 5, and 10 kV.

Swelling measurements

The swelling experiments were carried out by immersion of hydrogels specimen (9.0-mm diameter and 4.0-mm height) in distilled water. At definite intervals of time, the samples were removed from water and wiped with bolting paper to eliminate the excess water. The measurements were iterated until the hydrated gels achieved a constant weight value. The weight swelling percentage (Wp) for each sample was calculated as: $Wp = (Ws - Wd)/Wd \times 100$; where Ws is the weight of the swollen gel and the Wd is the original weight of the gel after polymerization.

Release studies

The matrix hydrogels and NCHGs containing CHX (cylindrical geometry 9 mm×4 mm) were prepared for release studies with the composition summarized in Table 1. The main purpose of these experiments was examined the release rate of the drug from the loaded matrices. For this purpose, the NCHG matrices were loaded with 15 mg of CHX, the NPs were loaded with 45 mg of CHX. The NCHGs were loaded with a total of 60 mg of the model drug. The investigated samples were immersed in distilled water (35 ml) and subjected to continuous magnetic stirring. At regular time intervals, an aliquot of 0.5 ml was removed, and the concentration of CHX was measured by HPLC.

Measurements of drug concentration by HPLC

The concentration of the released CHX was determined by HPLC on a Merck-Hitachi LaChrom instrument using C18 column, and UV detection at 257 nm. The mobile phase was 35% acetonitrile and 65% 20 mM acetate buffer with pH=3.8, and the flow rate was 0.5 ml/min.

Results and discussion

CHX has been used routinely to treat periodontal disease. However, when applied locally, it quickly spreads. By incorporating CHX into a polymer matrix, from which it can be slowly released, it can be expected to be more efficacious.

Preparation of nanoparticles

In this report, we describe the formation of NPs with a composition of HEMA/PEGDMA=50:50 mol%. The cross-linking density of the NPs is variable, which may affect the rate of release; however, this effect will be examined in a further study. Here, the vinyl groups of HEMA were

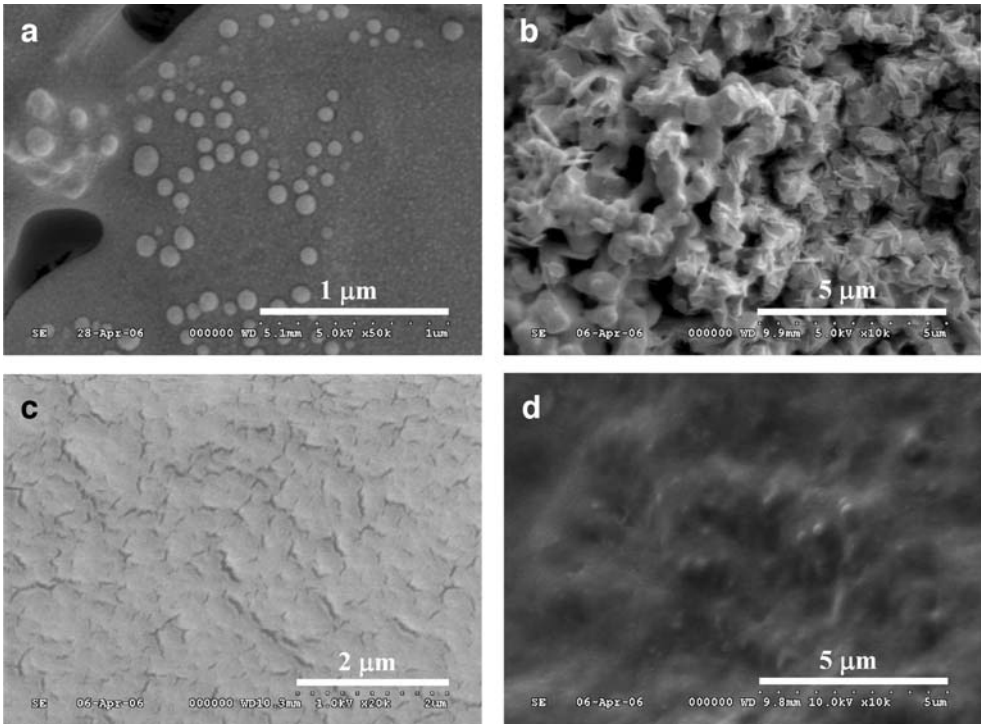

Fig. 1 SEM micrographs. **a** NPs of 5:5 HEMA/PEGDMA. **b** Matrix gel surface with a composition of HEMA/PEGDMA=5:5 (mold side surface), and **c** NCHG with a composition of 66% HEMA/PEGDMA=5:5 and 33% NPs (top side). **d** Broken NCHG of 66% HEMA/PEGDMA=5:5 and 33% NPs

crosslinked with the divinyl monomer of PEGDMA by free radical polymerization in micellar polymerization, forming stable NPs.

The particle size of HEMA–PEGDMA NPs was determined by scanning electron microscopy (SEM) and DLS measurements. SEM micrographs of crosslinked NPs of copolymer were taken from the colloid dispersion and freeze-dried form, using a concentration of 50 μg/ml. SEM micrographs (Fig. 1a) confirmed spherical, nanosized copolymer particles. The size of dried particles was in the range of 50–150 nm. The DLS measurements demonstrated that the NPs have a size distribution from 5 to 500 nm.

Synthesis of hydrogel and nanocomposite (photopolymerization)

The main objective of this work was to design CHX loaded NCHGs, which can release the drug in a slow manner compared to the matrix hydrogels. Because the inner structure, porosity of the gel is the most important parameter for the release properties, present hydrogels were investigated with different composition. The polymer solution weighed 2 g and five gels were prepared for parallel release measurements. The produced materials are yellow or white yellow soft and flexible hydrogels with cylindrical shape. The transparency was increased with increasing amount of PEGDMA.

In the NCHG, the amount of monomers in the hydrogel was constant. The NP/hydrogel and NP/monomer mass ratios were 0.15 and 0.5, respectively (Table 1). The composite gels were more compact than matrix gels and were more rigid and more transparent.

Mechanical assay

The assay of mechanical properties was repeated ten times to ensure reliable results. The matrix hydrogels were investigated in five different mol ratios (Table 2). When the amount of crosslinker was increased the compressive strength also risen. So this growing is depending on the cross-linker density and it could be in excess of 400% comparing sample 1 to sample 5. In the case of matrix gels, the compressive strength of sample 1 (HEMA/PEGDMA 9:1) changed from 0.18 MPa to 0.59 MPa for sample 3 (HEMA/PEGDMA 5:5) and than to 0.79 MPa for sample 5 (HEMA/PEGDMA 9:1). The compressive strength monotonously increases with the crossliking density. The com-

Table 2 The summary of results of mechanical assays of matrix hydrogel samples (1–5) and NCHG

Number of sample	Ratio of HEMA–PEGDMA	Percent strain (%)	Stress at maximum (MPa)
1	90:10	56.5	0.18
2	75:25	53.1	0.34
3	50:50	46.4	0.59
4	25:75	38.5	0.61
5	10:90	32.0	0.79
NCHG	50:50+30% NPs	25.3	0.56

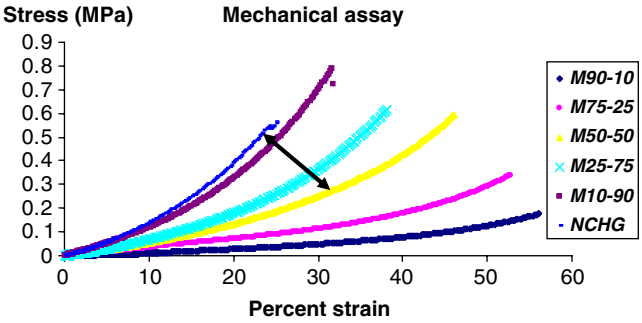

Fig. 2 The effect of the ratio of crosslinker in the matrix and that of the NPs for the compression properties of the hydrogels. *Black arrow* indicates two samples (M50:50 matrix and NCHG nanocomposite hydrogel) with the same composition of matrix gel (HEMA/PEGDMA=5:5; however, the NCHG sample consists of 30% of NPs)

pression strength values are very similar for the NCHG was 0.56 MPa compared to the 0.59 MPa value of the 50:50 matrix gel (sample 3). Nevertheless, the strain was altered accordingly because when the ratio of PEGDMA was only 10 and 25%, the samples were very soft, but when it was augmented (50, 75, or 90%), the specimens were brittle. The gels were harder and more rigid when the amount of crosslinker was increased. For the NCHG, it was observed that the compression strength value does not change related to the matrix; however, the flexibility decreases. It is caused by the nanoparticles, which are closer and harder than the matrix so they can reinforce it (the slope of curve of NCHG is higher at each point), but when they were mixed to the hydrogels, they become inhomogeneous and they can fracture easier. The shape of compressive strengths values is shown in Fig. 2.

SEM analysis

SEM micrographs of hydrogels, NPs, and NCHGs are shown in Fig. 1a–d. The picture of the hydrogel shows evidence those two types of gel surfaces are observed on the gel (Fig. 1b). These types do not differ severely from each other, and form the entire matrix. Either a more angular part is pronounced as angled shapes (right side of the picture above the scale bar), or it is embedded in another rounded environment (it is only a little part of this image on the left corner). This dual disposition is generally representative for all of the matrix.

SEM image of the surface of the NCHG is shown in Fig. 1c. The picture taken with 20,000 magnification shows a relatively smooth surface where only narrow cracks can be observed. The size of these cracks are from 100 to 200 nm but could not discovered other formations. The broken sample is analyzed in Fig. 1d; this picture shows large number of NPs in the matrix. This image shows particles with a size of about 200 nm ball-shaped nanoparticles inside the matrix.

Swelling ratios

This swelling procedure was studied for five parallel samples in case of hydrogel and in three for the NCHG.

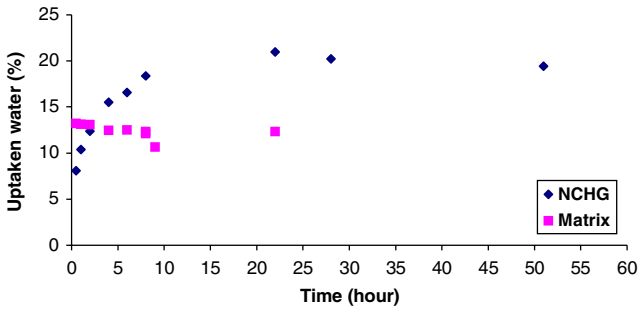

Fig. 3 The swelling properties of the 50% HEMA 50% PEGDMA hydrogel (matrix), and the NCHG composed of 66% 50:50 HEMA/PEGDMA hydrogel and 33% of 50:50 HEMA/PEGDMA NPs

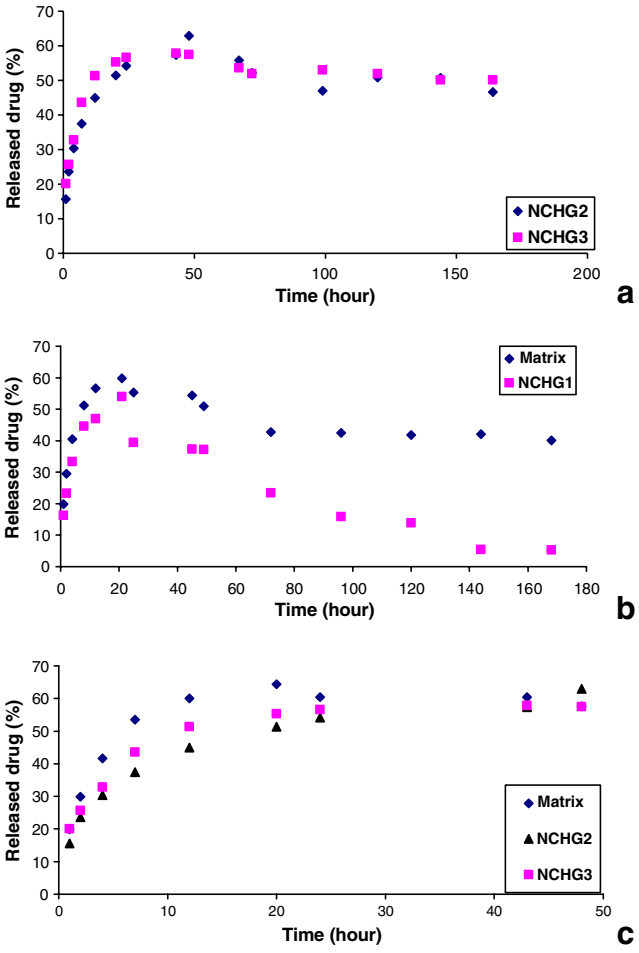

Fig. 4 Release profiles of matrix gel (50:50 HEMA/PEGDMA), and the NCHG (66% of 50:50 HEMA/PEGDMA hydrogel and 33% of 50:50 HEMA/PEGDMA NPs)

The representative swelling kinetic curves for hydrogels and NCHGs are shown in Fig. 3. The curves show that the weights of hydrogels after photopolymerization do not increase notably, it is about 13% in the first half hour and later an equilibrium state was observed. In contrast, the swelling behavior of NCHGs is different. It swelled slower in the first 2 h, but after this, the swelling was continued until 22 h when the samples have reached equilibrium weight. It seems that the final swelling capacity of the NCHG is higher (about 21%), but the water uptake is slower comparing to the matrix hydrogel. This remarkable swelling rate would be a favorable property, these kinds of materials can be usable for dental applications.

Release properties

The release curves of CHX from basic hydrogel (50% HEMA and 50% PEGDMA) and from the NCHGs are shown in Fig. 4. The release profiles were investigated in the case of matrix and for the NCHGs in separated reservoir. The measurements were performed for three parallel experiments to ensure reliable results. In the first 4 h, the difference was not too considerable, but after the seventh hour, a remarkable difference was observed. The release from the matrix gel was faster than the NCHGs where only the matrix was loaded (Fig. 4a). Accordingly, the effect of NP could be followed in the initial period. This controlled release was continued to 48 h after the degree of delivered drug approaches constant value (Fig. 4b, and extended part in Fig. 4c). The maximum ratio of the released drug in each case reached up to 60% of loaded drug, the main difference is altogether the time which it is eventuating. The NCHG1 sample with loaded matrix and empty NPs shows an unexpected profile. In the first period, CHX is released, but then its concentration declines. The NPs entrapped a part of the drug. The reason of this phenomenon has not been understood yet.

Conclusions

Nanocomposite hydrogels were successfully prepared by incorporation of nanoparticles into the gel matrix. The integrated gel system showed distinct advantages compared to simple hydrogels as drug delivery systems. The swelling ratio of NCHG is up to 200% related to the solid content and results in a flexible, Soft gel for implantation into the periodontal pockets or for application as a surface film on infected gums. The compression strength increased with higher content of the cross linker, PEGDMA. Adding NPs to the matrix this value remains constant. The release slope of CHX declined for NCHG, indicating a slower release of the drug from the composite hydrogels. Decrease of the CHX concentration can be observed in all the samples as it is shown in the figures. The decrease is largest for NCHG1, but in the other samples there is also a 10–15% CHX concentration decrease. The lower decrease could be from a small-scale decay of the drug, or that new places get free, where the CHX molecules can adsorb. The case of NCHG1 is more complicated. There is a system of three components, one is the NPs, closer around by the polymer matrix, and this complete tablet is merged in distilled water. There could be some equilibrium that was set in different speed; this is fast between the water and the matrix but slow among of the NPs, matrix, and the water. Therefore, it could be the reason why it goes out the drug fast to the release medium and why it goes back to the NPs after that. These results will be further investigated. In situ polymerization of hydrogels offers flexibility for local placement of drugs in the treatment of periodontal disease.

Acknowledgment This work was supported by GND (Grant of Genome Nanotech Debrecen): contract numbers 06/432/2004 and 06/423/2004.

References

1. Zrinyi M (1997) Trends in Polym Sci 5:280
2. Gispet J, Sola R, Varon C (2000) Cont Lens Anterior Eye 23:16
3. Hsiue G-H, Guu J-A, Cheng C-C (2001) Biomaterials 22:1763
4. Jankunas V, Rimdeika R, Pilipaityte L (2004) Medicina (Kaunas) 40:429
5. Heasman PA, Heasman L, Stacey F, McCracken GI (2001) J Clin Periodontol 28:90
6. Uhrich KE, Scott MC, Robert SL, Kevin MS (1999) Chem Rev 99:3181
7. Peppas NA, Bures P, Leobandung W, Ichikawa H (2000) Eur J Pharm Biopharm 50:27
8. Das A, Wadhwas S, Srivastava AK (2006) Drug Deliv 13:139
9. Lee D-Y, Spangberg LSW, Bok Y-BB, Lee C-Y, Kum K-Y (2005) Oral Surg Oral Med O 100:105
10. Jain A, Kim Y-T, McKeon RJ, Bellamkonda RV (2006) Biomaterials 27:497
11. Riggs PD, Braden M, Patel M (2000) Biomaterials 21:345
12. Huang CW, Sun YM, Huang WF (1997) J Polym Sci, Part A: Polym Chem 35:1873
13. Podual K, Doyle FJ III, Peppas NA (2000) Biomaterials 21:1439
14. Atkins TW, McCallion RL, Tighe BJ (1995) J Biomed Mater Res 29:291
15. Anseth KS, Scott RA, Peppas NA (1996) Macromolecules 29:8308
16. Friedman M, Golomb G (1982) J Periodontal Res 17:323
17. Steinberg D, Friedman M, Soskolne A, Sela MN (1990) J Periodontol 61:393
18. Lu S, Anseth KS (1999) J Control Release 57:291
19. Yue IC, Poff J, Cortes ME, Sinisterra RD, Faris CB, Hildgen P, Langer R, Shastri P (2004) Biomaterials 25:3743
20. Leung D, Spratt DA, Pratten J, Gulabivala K, Mordan NJ, Young AM (2005) Biomaterials 26:7145
21. Bako J, Szepesi M, Veres AJ, Borbely ZM, Hegedus Cs, Borbely J (2006) Polym Mat: Sci & Eng 94:367
22. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE (2001) J Control Release 70:1

23. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM (2004) J Control Release 100:5
24. Xia X, Hu Z, Marquez M (2005) J Control Release 103:21
25. Chung Y-I, Tae G, Yuk SH (2006) Biomaterials 27:2621
26. Kim D-H, Martin DC (2006) Biomaterials 27:3031
27. Koo OM, Rubinstein I, Onyuksel H (2005) Nanomedicine 1:193
28. Murugan R, Ramakrishna S (2005) Compos Sci Technol 65:2385
29. Sinha A, Das G, Sharma BK, Roy RP, Pramanick AK, Nayar S (2007) Mat Sci Eng C 27:70
30. Weian Z, Wei L, Yue'e F (2005) Mat Lett 59:2876
31. Hussein MZ, Yahaya AH, Zainal Z, Kian LH (2005) Sci Techn Adv Mat 6:956