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Design and In Vitro Evaluation of Polymeric Formulae of Simvastatin for Local Bone Induction

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ABSTRACT Simvastatin (SVS), a cholesterol-lowering drug, has been shown to stimulate bone formation. This study deals with the design and in vitro evaluation of local delivery systems for simvastatin. They are intended to treat bony defects resulting from periodontitis or to induce osteogenesis around the titanium implants. Granules and gels were formulated using bioerodible/biocompatible polymers, namely hydroxypropylmethyl cellulose (H), sodium carboxymethyl cellulose (C), and chitosan (Ch). The in vitro release profiles and kinetics were evaluated and the swelling and/or erosion was monitored. Differential scanning calorimetry (DSC) and infrared (IR) were used to detect any SVS/polymer interactions that may affect drug release. The results revealed variable extents of controlled drug release from the designed formulae depending on the polymer nature. About 50% cumulative SVS was released from both H granules and gel formulae within 24 h and ~66% and ~88% from C granules and gel, respectively. Ch formulae exhibited ~50% release from granules and ~30% from gel.

KEYWORDS Simvastatin, Local delivery, Osteogenesis, Granules, Gel, In vitro release

INTRODUCTION

Periodontal disease alters bone morphologic features and reduces bone height. The stimulation of local bone formation is an important factor in the repair of isolated bony defects resulting from periodontitis (Thylin et al., 2003). Osteogenesis around the titanium implants is also needed to ensure prompt and complete osseointegration of the dental implants (Ayukawa et al., 2004).

Most bone-modulating drugs currently used as biphosphonates, calcitonin, estrogen, selective estrogen receptor modulators, and vitamin D analogues inhibit bone resorption instead of primarily stimulating new bone formation (Garret et al., 2001). Bone-forming agents are capable of rebuilding bone; among these anabolic agents, recombinant bone morphogenetic protein-2 (BMP-2) was reported (Saito et al., 2001). Oral bone induction by local BMP-2 in humans has been variable (Howell et al., 1997). In addition, concern has

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been raised about the expense and drug stability in such protein therapy, as well as the possibility of eliciting an antibody response (Garret et al., 2001).

To discover small molecules that induce BMP-2, Mundy et al. (1999) examined more than 30,000 compounds and tested their effects on BMP-2 gene expression. They identified that statins specifically increased expression of the BMP-2 gene. In addition, statins possess an antiresorptive effect, as they interfere with the mevalonate pathway leading to inhibition of osteoclast activity and osteoblast apoptosis (Edwards & Spector, 2002). Statins may also affect bone formation indirectly by inhibiting inflammation and promoting angiogenesis and vascular invasion (Edwards & Spector, 2002). The bone inductive effect of both locally and orally administered statins was evaluated in rodents (Mundy et al., 1999), and the results suggested that statins, if selectively targeted to bone, will have beneficial effects in the treatment of osteoporosis and bony defect.

Statins mostly exist as prodrugs that must be converted to active forms. They are targeted to liver and not to bone. Most are lipid soluble, which means that they enter cells easily. From the point of view of possible osteogenic effect of statins, "getting into the bone cells is what it is all about" (Watts, 2002). Some of the newer statins, particularly pravastatin and robuvastatin, are water soluble and depend on specific carrier mechanism to enter the liver cells. It is unlikely that this same carrier mechanism is present in the bone cells. Those hydrophilic statins failed to show ostegenic effect (Watts, 2002). The most efficacious statins would be those that distribute themselves to the bone or bone marrow (Mundy et al., 1999). Statins have clear effects on bone formation in vitro, but the formulation of existing "liver-targeted" statins requires further refinement for efficacy in vivo (Von Stechow et al., 2003). The more recent potent statins such as cerivastatin may get pass the liver and reach the bone (Garett & Mundy, 2002).

Periodontal therapy would necessitate targeting to specific defects, suggesting the importance of local application. The effects of simvastatin gels on murine calvarial bone indicated that a single, high dose of simvastatin in methyl cellulose gel, particularly under an occlusive membrane, can stimulate new cranial bone (Thylin et al., 2003). Wong and Rabie (2003, 2005) showed that simvastatin in collagen matrix, when grafted to bony defect, induced an accelerated formation of bone locally and triggered the early expression of growth factors to regulate angiogenesis, differentiation of bone cells, and osteogenesis. Multiple injections of simvastatin for a 5-day period over mouse calvaria resulted in a 30% to 50% increase in bone thickness and area (Garett & Mundy, 2002). Whang et al. (2005) determined the effect of grafting statins to polylactide-co-glycolide (PLG) on bone regeneration in vitro; the results showed enhanced bone cell mineralization.

This study focused on the development and in vitro evaluation of local delivery systems containing simvastatin. Published in vitro and in vivo results have shown that simvastatin is among the most potent in stimulating bone growth, mainly when locally targeted (Thylin et al., 2003). Hydroxypropylmethyl cellulose, sodium carboxymethyl cellulose, and chitosan were used as example of non-ionic, anionic, and cationic carriers for simvastatin in granules or gel formulations.

EXPERIMENTAL

Materials

Simvastatin (SVS), (Merck Sharp & Dohme, Whitehouse Station, NJ, USA), was a kind gift from Pharco Pharmaceuticals Co. (Alexandria, Egypt). Hydroxypropylmethyl cellulose 4000 cp (H) and sodium carboxymethyl cellulose (C) were supplied from Alexandria Pharmaceuticals Co. (Alexandria, Egypt). Chitosan (Ch), a high purity powder from crab shells having maximum granule size of 0.2 mm and degree of deacetylation $>$ 80%, was obtained from CarboMer, Inc. (San Diego, CA, USA). Other chemicals were of analytical grade.

METHODS

Formulae Preparation

Granules were prepared to contain 2.2 mg of SVS per 150 mg total weight. The reported local SVS dose was 2.2 mg (Thylin et al., 2003) and the preliminary clinical study revealed that 150-mg granules were suitable for surgical grafting.

Weighed SVS/polymer blends were accurately mixed and wetted, forming a mass to be forced through a sieve. The obtained granules were sized, dried in a circulating air oven at 60°C, then subjected to vacuum and stored in a desiccator until use.

The choice of a wetting agent depends on the polymer characteristics (Schott, 1993); to avoid lumping and to ensure adequate wetting, the solvent should be conducive to limited swelling and poor dissolution. For H granules, the wetting system was a 4:1 isopropyl alcohol/water mixture, and a 1:1 mixture was used to prepare C granules. Lactic acid (1%) was the wetting agent for Ch granules.

The obtained granules ranged from 800 to 1000 μm except for Ch granules (1000–1500 μm), as sizing of the wetted Ch mass necessitated a larger mesh size sieve.

The H gel (8% w/v) was prepared by adding polymer gradually to 1/3 the volume of distilled water maintained at 90°C with gentle stirring, then completed to volume with cold distilled water. The C gel (8% w/v) and Ch gel (4% w/v) were prepared by dispersing the polymer gradually in distilled water or 1% lactic acid, respectively, with gentle stirring. The obtained polymeric dispersions were subjected to overnight refrigeration, resulting in clear gels. The calculated amount of SVS was dispersed into the gel systems before the release run (2.2 mg/300 mg sample). Preliminary study showed that the consistency of the obtained gels as well as the sample size were suitable for surgical insertion.

Solubility Determination

Excess SVS powder was placed in 10 mL of dissolution medium composed of isotonic phosphate buffer (PBS), pH 7.4, containing 0.5% sodium dodecyl sulfate (SVS tablet monograph, USP/NF-23). The container was subjected to 12-h shaking at 37°C followed by overnight equilibrium (37°C). The saturation solubility was found to be 1831 mg/mL.

In Vitro Release

The granules (150 mg) were placed in polyester gauze to prevent their dispersion in the medium during release (simulating "packing" at the surgical insertion sites).

The gels (300 mg) were filled into a circular stainless-steel cup (14 mm i.d. and 1 mm depth) and covered with polyester gauze. The gauze acts as a mechanical barrier preventing granule dispersion or gel escape without interfering with drug release.

The formulae, granules or gels, were introduced into 10-mL beakers, and 5 mL of release medium (PBS, pH 7.4, containing 0.5% sodium dodecyl sulfate at 37°C) was layered on the samples. The release systems were subjected to horizontal shaking in a thermostatically controlled water bath (30 end-to-end shaking rate). The 5 mL of release medium was taken as a sample at a specified time interval and replaced by a fresh 5 mL of medium at 37°C. The samples were assayed spectrophotometrically at λ_{max} 258 nm using a Jenway 6305 UV/Vis spectrophotometer (UK). Both the lactone form (SVS) and the β-hydroxy form (SVS-OH) were evaluated (Ellison et al., 1993). Release media resulting from drug-free formulae were used as blanks to omit any interference from the polymers used. An average of 3 determinations were calculated.

Weight Change Monitoring

Weight changes of the formulae were monitored parallel to the in vitro SVS release (average of 2 determinations). The formulae were blotted between 2 filter papers and weighed after each removal of the release medium.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry measurements were performed using a Perkin Elmer Pyris Series DSC6 (USA). The DSC curves of SVS (2 mg) or 1:1 SVS/polymer blends (4 mg) were obtained by heating the samples from ~25°C to ~200°C at a heating rate of 2°C/min under nitrogen atmosphere.

Infrared

The infrared absorption spectra of SVS and SVS/polymer blends as KBr pellets were obtained using a Perkin Elmer Spectrum RXIFT IR system (USA).

RESULTS AND DISCUSSION

The lactone form of simvastatin (SVS) is a prodrug targeted to the liver, where it is transformed to the active β-hydroxy acid form (SVS-OH). The latter interferes with the mevalonate pathway, an early rate-limiting step in cholesterol biosynthesis (Ellison et al., 1993). The evaluation of the locally targeted SVS did not reveal any predominance of SVS-OH over SVS

regarding their osteoinductive effect (Thylin et al., 2003; Wong & Rabie, 2003, 2005). In this study, the drug released from the designed local delivery systems was evaluated using a UV assay, which measured the drug in either form (Ellison et al., 1993).

The design of the in vitro release experiment aimed to simulate in vivo conditions (surgical grafting to bony defect). The sample was "packed" and subjected to gentle shaking in 5 mL of PBS, pH 7.4, at 37°C. The addition of sodium dodecyl sulfate was to improve SVS solubility and to achieve sink condition (saturation solubility was 1831 mg/mL). Sink condition was further maintained by removing the whole volume as a sample and replacing it by fresh medium.

Figure 1A depicts the SVS released from granule formulae; 48.66%, 66.33%, and 49.17% were released from H, C, and Ch granules, respectively, after 24 h. Gel formulae showed different release patterns; 55.46% was obtained from H, 88.08% from C, and 30.49% from Ch gels after 24 h (Fig. 1B).

Monitoring the percent change in granule and gel weight (Fig. 2) reflected the water uptake (swelling) and/or erosion of the formulae during the 24-h release run. The water uptake by H and C granules induced about 400% and 700% initial weight increase followed by a gradual decline (~38% for H and ~158% for C after 24 h) (Fig. 2A). Significant initial swelling (>800%) was observed for chitosan granules, which persisted for almost 24 h (Fig. 2A). Regarding gel formulae, the Ch gel showed an approximate 50% weight increase over 6 h, which declined to 35% after 24 h (Fig. 2B). Both H and C gel formulae showed initial swelling followed by erosion after about 3 h (Fig. 2B).

Hydrophilic polymers are entangled giant molecules. When they come in contact with water molecules, forces of attraction, chiefly hydrogen bonding, break up polymer-polymer contact, leading to swelling and increase in size (Wan et al., 1991). Swelling of the hydrocolloid systems plays an essential role in the drug release, and numerous methods have been developed to document these events such as recording dimensional changes and using a photo-video camera in association with computer image analysis (Dorozynski et al., 2004).

Rapid water uptake was observed for H formulae (Fig. 2A and B), followed by erosion of the system starting after 6 h for the granules and after 2 h for the gel. The 6-h release profile of either granules or gel showed more than 50% cumulative SVS released. This limited result agrees with the hydrodynamic explanation of water/H systems (Wan et al., 1991); the molecular mobility within the swollen hydrated H systems is restricted due to the viscous drag, which is sufficient to oppose the driving force influencing drug release. Lower H content (gel formula compared

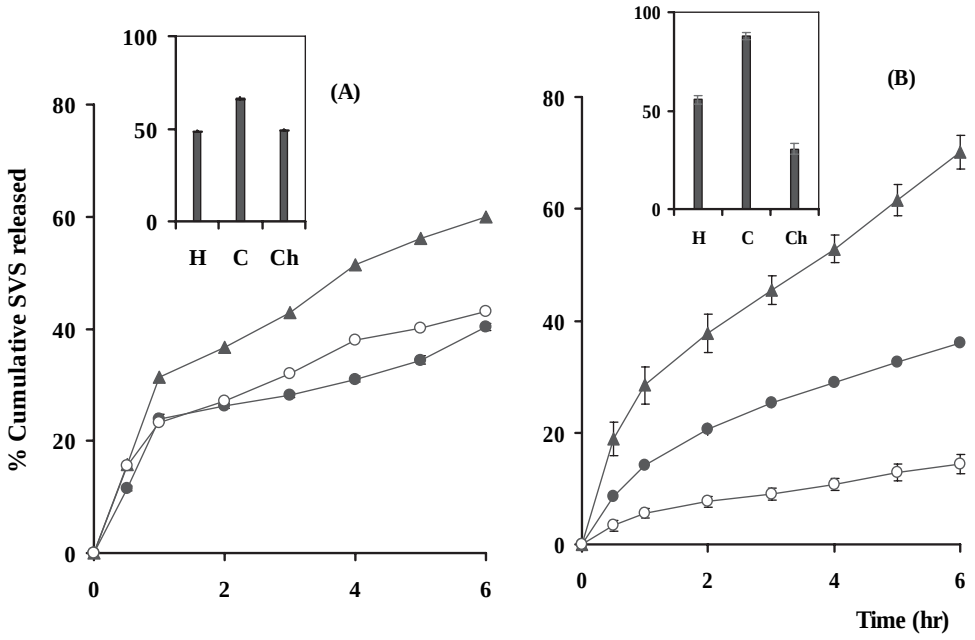

FIGURE 1 Cumulative Percent SVS Released From (•) H, (▲) C, and (○) Ch Granules (A) and Gels (B). The Inserts Represent the Cumulative Percent Released After 24 h.

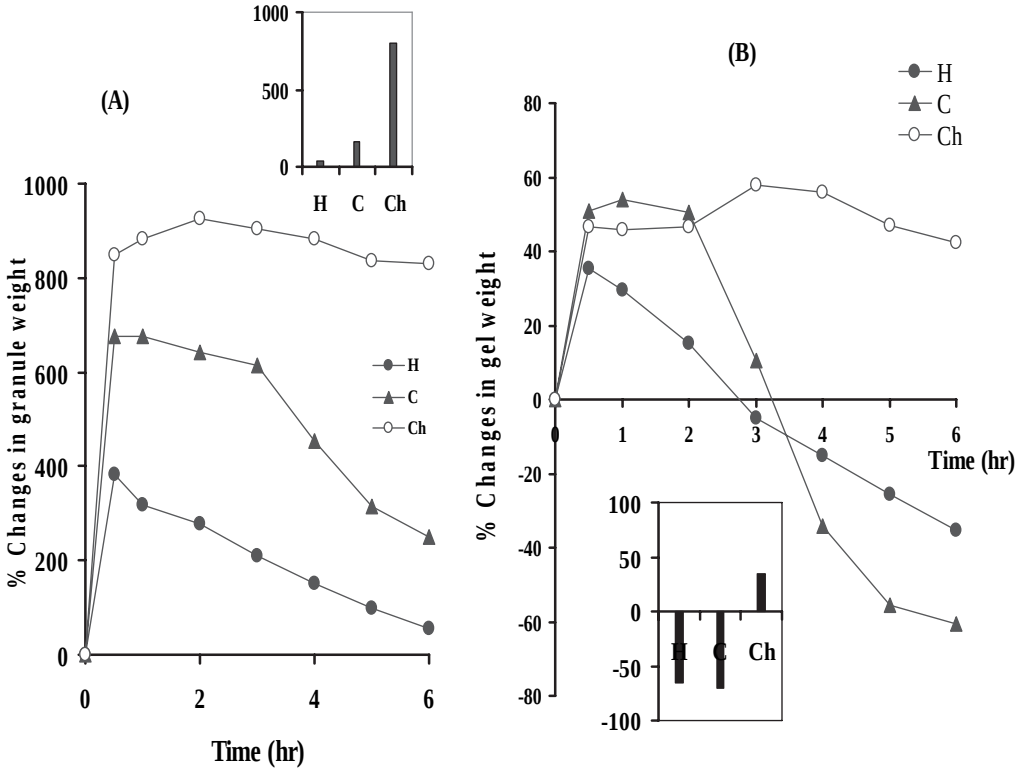

FIGURE 2 **Percent Weight Changes in Granules (A) and Gels (B) During Exposure to the Release Medium at 37°C. The Inserts Represent the Percent Changes After 24 h.**

to granule formula) did not reflect on SVS release profile (Fig. 1A and B); the viscous force opposing drug mobility appears to be a common factor in both formulae.

In addition to its hydrophilic swellable characteristics, C is ionized and expands at pH 7.4, inducing more water penetration (Boraie et al., 1990). SVS release was faster through C systems compared to H systems having the same drug polymer ratio (Fig. 1A and B). A faster erosion rate of C formulae confirmed faster release compared to H formulae (Fig. 2A and B). These results were in agreement with previous comparative studies evaluating release from H and C systems (Devi et al., 1989; Boraie et al., 1990).

Ch is a naturally occurring polysaccharide prepared from chitin of crabs and lobsters by N-deacetylation with alkali. Ch was useful for the preparation of sustained release indomethacin granules (Hou et al., 1985). Swelling of Ch granules was monitored and related to pH of the medium as well as the indomethacin release; increased swelling at the low pH would facilitate the movement of drug molecules out of the granules (Hou et al., 1985). An approximate 700–900% increase in granule weight over 24-h exposure to the release medium was observed for Ch (Fig. 2A). This was accompanied by an approximate 50% cumulative SVS released (Fig. 1A). In spite of the appreciable water uptake of the Ch granules, they remained rigid. Excessive swelling of the Ch granules may restrict their use as local delivery for SVS; they will be unsuitable for surgical grafting unless the polymer content is lowered. The weight increase of Ch gel ranged from 35% to about 60% during the 24-h exposure to the medium, indicating persistence of the gel integrity compared to the cellulose gel formulae (Fig. 2B). The influence of pH on the swelling of Ch gels and drug release from gels was studied (Aksungur et al., 2004). It was shown that the swelling properties and drug release from gels were increased under acidic conditions due to protonation of amino groups of Ch. Figure 1A and B revealed the superiority of SVS release from Ch granules compared to the gel, about 50% cumulative release from granules compared to about 30% from gel after 24 h.

The kinetic analysis of the release profiles was carried out according to the general Peppas equation (Peppas, 1985) (Table 1). The results indicated

TABLE 1 **Release Kinetic Parameters[a] and Percent Dissolution Efficiency (% DE) of SVS From Different Formulae**

Formula	K^b	n^c	R^d	% DE
Granules				
H	0.193	0.340	0.931	40.005
C	0.276	0.362	0.920	57.743
Ch	0.224	0.306	0.953	42.277
Gels				
H	0.140	0.485	0.985	40.173
C	0.284	0.417	0.974	70.304
Ch	0.052	0.555	0.998	19.024

[a]According to the general Peppas equation $M_t/M_\infty = Kt^n \cdot M_t$ is the cumulative amount of drug released at time t, M_∞ is the total amount of drug incorporated.

[b]K is a constant reflecting structural and geometric characteristics of the release system.

[c]n is the release exponent, indicative of the mechanism of drug release.

[d]R is the linear regression value of the logarithmic form of the equation.

combined diffusion mechanisms for granule systems ($n < 0.5$); diffusion was partially through a swollen matrix and partially through water-filled pores. Regarding the gel formulae, Fickian diffusion was observed ($n \sim 0.5$), indicating that the release rate was dependent on $t^{-0.5}$.

Percent dissolution efficiency (% DE, Table 1) was calculated for granules and gels (Varshosaz et al., 2002). The results indicated again similarity of SVS release characteristics from both H formulae. Faster erosion of C gel compared to the granules reflected higher % DE (Table 1). On the contrary, the % DE obtained from Ch gel was about half the value obtained from Ch granules; this may be due to the positive influence of the excessive water drag inside the granules, allowing better mobility of drug molecules in a diluted environment. In addition, excessive water may induce dissociation of the SVS/Ch combination, if any (Fig. 2A and B).

Differential scanning calorimetry analysis (DSC) was performed to detect the occurrence of SVS/polymer interaction (Fig. 3 and Table 2). Slight changes were observed regarding the temperature range and the peak temperature except for the SVS/Ch combinations, which showed early peak onset for both physical mixture and lactic acid-treated blend; significant broadening was observed in the latter case with significant change in peak temperature. On comparing enthalpy values of SVS and SVS/polymer systems, deviation was observed in the presence of polymers; this deviation was pronounced in the SVS/Ch lactic acid-treated sample. The slope ratio values (Kim et al., 1985) confirmed that Ch influenced the thermal behavior of SVS mainly when it was lactic acid treated.

Being polyhydroxylated, the cellulose derivatives H and C may interact with SVS via hydrogen bonding. No probable ionic interaction between the anionic C and SVS or SVS-OH is expected.

Regarding SVS/Ch systems, the presence of lactic acid induced protonation of the amino groups and encouraged reversible conversion of SVS to SVS-OH (Elliso et al., 1993). Probable ionic interaction is expected according to the pH of the medium.

Infrared (IR) absorption spectra of SVS, Ch, SVS/Ch physical mixture, and SVS/Ch lactic acid-treated blend were compared. Complete disappearance of the carbonyl lactone stretch (1698 cm^{-1}) was observed in SVS/Ch lactic acid-treated blend spectrum with the emergence of a peak at 1639 cm^{-1} (free carboxylic group). This indicated opening of the lactone ring of SVS. During release, at pH 7.4, both amino groups (Ch) and carboxylic group (SVS-OH) might be ionized to a certain extent, allowing ionic combination. This may explain the delayed release, mainly from the Ch gel. As mentioned earlier, the water drag was less pronounced in the Ch gel compared to the Ch granules (Fig. 2A and B); excessive swelling and dilution might weaken ionic combination in granule system.

In a step toward clinical application of the designed SVS formulae, the in vivo performance of surgically inserted SVS/H granules in bony defects was assessed (Rady, 2005). A 9-month study, comprising clinical and radiological evaluation, was performed and the outcome was monitored in 10 periodontal patients. The results revealed statistically significant clinical improvement compared to a control group treated only with surgical debridement. The radiological data showed a significant increase in bone mineral density. Furthermore, the histological influence of surgically inserted SVS/H granules was assessed in five dogs with surgically induced bony defects. The results confirmed the osteoinductive effect of the locally applied SVS formula (Rady, 2005).

Additional cooperative study (Department of Oral Surgery, Faculty of Dentistry, Alexandria) is ongoing

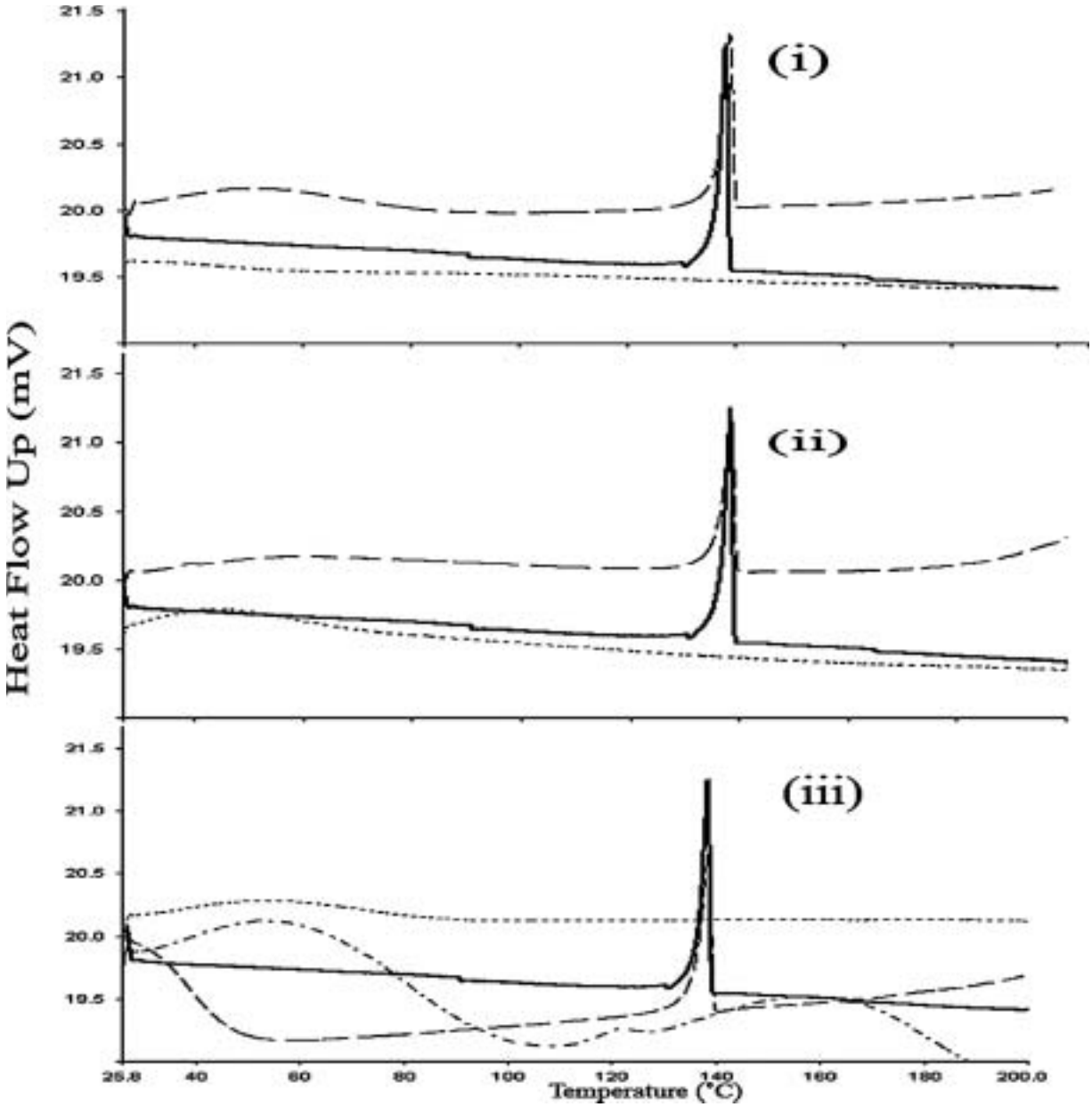

FIGURE 3 **Differential Scanning Thermograms of SVS (——), Polymer (· · · ·), and 1:1 Physical Mixture (– – –); (i) Shows the Influence of H on SVS Thermal Characteristics, (ii) the Influence of C, and (iii) that of Ch. The Dashed Dot Line (–· –· –·) Represents the Thermogram of the Lactic Acid-Treated SVS/Ch Blend.**

TABLE 2 **DSC Data for SVS and SVS/Polymer Blends (1:1)**

Sample	Melting range (°C)	Peak temp. (°C)	Peak height (mW)	ΔH J/g	Peak slope ratio
SVS	136.88–139.15	138.45	1.70	53.52	2.40
SVS/H	136.87–139.97	139.03	1.30	25.94	2.27
SVS/C	136.11–139.42	138.48	1.13	23.77	2.42
SVS/Ch[a]	135.77–139.50	138.47	1.27	29.10	2.82
SVS/Ch[b]	135.04–181.65	161.28	0.36	90.41	0.80

[a](1:1) Physical mixture.
[b](1:1) Blend, treated with few drops of 1% lactic, then dried at 60°C and stored overnight in vacuum.

to investigate the osteogenic effect of SVS/H gel formula around titanium implants.

CONCLUSION

Local SVS delivery formulae were designed using bioerodible/biocompatible polymers. The release from H formulae was more prolonged than from C formulae; faster erosion of C formulae contributed to their faster release. The Ch formulae showed a minimum tendency for erosion, indicating probable prolonged residence in vivo. The slow in vitro release of SVS from the Ch gel confirmed its suitability for long-term treatment, but the observed excessive swelling of Ch granules may restrict their use for surgical application to bony defects.

In vivo results (Rady, 2005) showed the suitability of SVS/H granules as an osteogenic agent. Further studies in collaboration with the Faculty of Dentistry are planned for clinical evaluation of C and Ch formulae.

REFERENCES

Thylin, M. R., Mc Connell, J. C., Schmid, M. J., Reckling, R. R., Ojha, J., Bhattacharyya, I., Marx, D. B., & Reinhardt, R. A. (2003). Effects of simvastatin gels on murine calvarial bone. *J. Periodontol., 73*, 1141–1148.

Ayukawa, Y., Okamura, A., & Koyano, K. (2004). Simvastatin promotes osteogenesis around titanium implants. *Clin. Oral Implant. Res., 15*, 346–350.

Garret, I. R., Gutierrez, G., & Mundy, G. R. (2001). Statins and bone formation. *Curr. Pharm. Des., 7*, 715–736.

Saito, N., Okada, T., Horiuchi, H., Murakami, N., Takahashi, J., Nawata, M., Ota, H., Miyamota, S., Nazaki, K., & Takaoka, K. (2001). Biodegradable poly D-L-lactic acid-polyethylene glycol block copolymers as BMP delivery system for inducing bone. *J. Bone Joint Surg. Am., 83* (A Suppl), S92–S98.

Howell, T. H., Fiorellini, J., Jones, A., Alder, M., Nummikoski, P., Lazaro, M., Lilly, L., & Cochran, D. (1997). A feasibility study evaluating rh BMP-2/absorbable collagen sponge device for local alveolar ridge preservation or augmentation. *Int. J. Periodont. Restor. Dent., 17*, 124–139.

Mundy, G., Garett, R., Harris, S., Chan, J., Chen, D., Rossini, G., Boyce, B., Zahao, M., & Gutierrez, G. (1999). Stimulation of bone formation in vitro and in rodents by statins. *Science, 286*, 1946–1949.

Edwards, C. J., & Spector, T. D. (2002). Statins as modulators for bone formation. *Arthritis Res., 4*, 151–153.

Watts, N. (2002). Biophosphonates, statins, osteoporosis and atherosclerosis. *South. Med. J., 95*, 578–582.

Von Stechow, D., Fish, S., Yahalom, D., Bab, I., Chorev, M., Muller, I., & Alexander, J. M. (2003). Does simvastatin stimulate bone formation in vivo? *BMC Musculoskelet. Disord., 4*, 1–8.

Garett, J. R., & Mundy, G. R. (2002). The role of statins as potential targets for bone formation. *Arthritis Res., 4*, 237–240.

Wong, R. W. K., & Rabie, A. B. M. (2003). Statins collagen grafts used to repair defects in the parietal bone of rabbits. *Br. J. Oral Maxillofacial Surg., 41*, 244–248.

Wong, R. W. K., & Rabie, A. B. M. (2005). Early healing pattern of statin- induced osteogenesis. *Br. J. Oral Maxillofacial Surg., 43*, 46–50.

Whang, K., Grageda, E., Khan, A., McDonald, J., Lawton, M., & Satsangi, N. (2005). A novel osteotropic biomaterial OG-PLG: in vitro efficacy. *J. Biomed. Mater. Res. A., 74A*, 247–253.

Schott, H. (1993). Polymer science. In *Physical Pharmacy* (4th Ed.); Martin, A., Bustamante, P., & Chun, A. H. C., Eds.; Lea and Febiger: Philadelphia; 556–594.

Ellison, D. K., Moor, W. D., & Petts, C. R. (1993). Simvastatin. In H. G. Brittain & K. Florey (Eds.), Analytical *Profiles of Drugs Substances and Excipients* (Vol. 22) (pp. 359–388). San Diego, CA: Academic Press, Inc.

Wan, L. S. C., Heng, P. W. S., & Wong, L. F. (1991). The effect of hydroxy- propylmethyl cellulose on water penetration into a matrix system. *Int. J. Pharm., 73*, 111–116.

Dorozynski, P., Jachowicz, R., Kulinowiski, P., Kweicinski, S., Szybinski, K., Skorka, T., & Jasinski, A. (2004). The macromolecular polymers for the preparation of hydrodynamically balanced systems-methods of evaluation. *Drug Dev. Ind. Pharm., 30*, 947–957.

Boraie, N. A., El-Khawas, M., & Naggar, V. F. (1990). A study of the prolonged release kinetics of soluble drugs from various hydrophilic directly compressed cellulose matrices. *S.T.P. Pharma., 6*, 6–12.

Devi, K. P., Ranga Rao, K. V., Baveja, S., Fathi, M., & Roth, M. (1989). Zero-order release formulation of oxprenolol hydrochloride with swelling and erosion control. *Pharm. Res., 6*, 313–317.

Hou, W.-M., Miyazaki, S., Takada, M., & Komai, T. (1985). Sustained release of indomethacin from granules. *Chem. Pharm. Bull., 33*, 3986–3992.

Aksungur, P., Sungur, A., Unal, S., Iskit, A. B., Squier, C. A., & Senel, S. (2004). Chitosan delivery systems for the treatment of oral mucositis: in vitro and vivo studies. *J. Control. Rel., 28*, 269–279.

Peppas, N. A. (1985). Analysis of Fickian and Non-Fickian drug release from polymers. *Pharm. Acta. Helv., 60*, 110–111.

Varshosaz, J., Tavakoli, N., & Saidain, S. (2002). Development and physical characterization of periodontal bioadhesive gel of metronidazole. *Drug Deliv., 9*, 127–133.

Kim, K. H., Frank, M. J., & Henderson, N. L. (1985). Application of differential scanning calorimetry to the study of solid drug dispersions. *J. Pharm. Sci., 74*, 283–289.

Rady, A. G. (2005). "Evaluation of the Local Application of Cholesterol Lowering Drug (Simvastatin) for the Management of Intrabony Defects." Master thesis in oral medicine, periodontology, oral diagnosis and radiology. Faculty of Dentistry, Alexandria University, Egypt.