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ORIGINAL ARTICLE

Local drug delivery system for the treatment of osteomyelitis: *In vitro* evaluation

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ABSTRACT

Local antimicrobial delivery is a potential area of research conceptualized to provide alternative and better methods of treatment for cases, as osteomyelitis where avascular zones prevent the delivery of drugs from conventional routes of administration. Drug-loaded polymers and calcium phosphates as hydroxyapatites have been tried earlier. Bioactive glasses are bone-filling materials used for space management in orthopedic and dental surgery. A new bioactive glass (SSS2) was synthesized and fabricated into porous scaffold with a view to provide prolonged local delivery of gatifloxacin and fluconazole as suitable for the treatment of osteomyelitis. The new SSS2 was characterized by Fourier transform infrared (FTIR) and X-ray diffraction (XRD) analyses. In addition, the bioactivity of the SSS2 glass and resulting scaffold was examined by *in vitro* acellular method and ascertained by FTIR and XRD. The pore size distribution was analysed by mercury intrusion porosimetry and the release of drugs from scaffolds were studied *in vitro*. The glass and the resulting scaffolds were bioactive indicating that they can bond with bone *in vivo*. The scaffolds were porous with pores predominantly in the range of 10–60 μm, released the drugs effectively for 6 weeks and deemed suitable for local delivery of drugs to treat osteomyelitis.

Keywords: Drug release, chitosan, bone infection, bioceramic, composite, bone implant, ceramic implants, gatifloxacin, skeletal drug delivery, fluconazole

Introduction

Local antimicrobial delivery is a potential area of research conceptualized to provide alternative and better methods of treatment for cases such as osteomyelitis in which avascular zones prevent the delivery of drugs from conventional routes of administration. Infective microorganisms cause osteomyelitis, an inflammatory process accompanied by bone destruction. Current treatment strategies involve surgical curettage of infected bone followed by parenteral administration of high doses of antimicrobials for 4–6 weeks (Soundrapandian et al., 2007; Ouédraogo et al., 2008) as causative organisms are known to produce large amount of glycocalyx material and necrotic bones providing a surface suitable for the development of biofilm (Itokazu et al., 1997). Prolonged hospitalization for parenteral administration makes the therapy further costly and inconvenient. Local drug delivery systems release drug locally for prolonged periods and at concentrations generally higher than those achieved by multidose parenterals (Kundu et al., 2010). Polymeric drug delivery systems that release drugs locally for prolonged periods is slowly being viewed as an alternate choice to treat osteomyelitis (Tian et al., 2002; Ouédraogo et al., 2008). However, in general, various factors such as nondegradability of polymers such as polymethylmethacrylate, cost of biodegradable polymers, tissue reactions, and or immunological considerations seriously restrict the choice (Soundrapandian et al., 2007).

Bioactive glasses are a group of biologically acceptable glasses used as bone-filling material in dental and orthopedic surgery (El-Ghannam et al., 2005). They are biocompatible, biodegradable, and cheaper than most synthetic biodegradable polymers, and they

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can also bond physically and chemically with bone (Soundrapandian et al., 2009). Bioactive glasses of certain compositions have been tried as glass disks (Zhao et al., 2008) and monoliths (Andrade et al., 2009) for drug release. However, drug release from these systems neared completion in a week, making their selection questionable in the treatment of osteomyelitis.

In this article, we report the development and *in vitro* characterization of a porous bioactive glass scaffold based on a new composition of bioactive glass, as a drug delivery system suitable for the treatment of osteomyelitis.

Materials and methods

Preparation and characterization of bioactive glass

Bioactive glass (SSS2) was prepared by conventional glass melting procedure at 1400°C in a platinum crucible with an oxide composition of 42.5%W/W SiO_2 (Merck, Darmstadt, Germany), 21.2%W/W CaO (Sigma-Aldrich, St Louis, MO), 26%W/W Na_2O (Merck, Mumbai, India), 7.8%W/W P_2O_5 (Merck, Darmstadt, Germany), and 2.5%W/W ZnO (Loba-Chemie IndoAustranal Co, Mumbai, India).

Characterization of SSS2

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectrum of SSS2 was recorded in an FTIR spectrophotometer (Spectrum 100, PerkinElmer, Waltham, MA). Powdered SSS2 glass was triturated with KBr and compressed into disc at 10-ton pressure to make fine homogeneous discs for spectroscopic studies. The spectrum was scanned over the wave number range of 4000–400 cm^{-1} at a resolution of 2 cm^{-1}.

X-ray diffraction spectroscopy

X-ray diffraction (XRD) analysis was performed with an X-Ray diffractometer (X'Pert Pro, Phillips Analytical, the Netherlands). Powdered SSS2 glass was scanned from 10° to 70° diffraction angle (2θ) range with monochromated Cu Kα1 radiation and at a scan speed of 2 m^{-1}.

Acellular in vitro *bioactivity*

Simulated body fluid (SBF) containing SSS2 powder in well-closed polystyrene bottles was maintained at 37°C. Samples were collected after 1, 3, and 7 days and analysed by FTIR.

Preparation of porous SSS2 scaffolds

SSS2 glass powder was mixed with equal quantity of porogen (naphthalene) and pressed into cylindrical blocks by cold-isostatic press (Epsi, Temse, Belgium) at 150 MPa. Subsequently, blocks were turned and cut into circular disks of various dimensions (Refer Table 1) with a low-speed saw (Isomet, Buehler, IL). The shaped samples were thereafter subjected to graded temperatures from room temperature to 80°C, and finally fired at about 725°C on a Pt-Rh plate and stored in a vacuum dessicator until further use.

Characterization of porous scaffolds

Porosity and pore size distribution studies

Porosity of the blocks was determined by water displacement method based on Archimede's principle and pore size distribution by using a mercury porosimeter (PM60, Quantachrome, FL).

Acellular in vitro *bioactivity of SSS2 scaffolds*

Acellular *in vitro* bioactivity of SSS2 scaffolds was evaluated in the same way as that of SSS2 glass. After definite time intervals, the SSS2 scaffolds were studied using scanning electron microscope (SEM) (Steroscan430i, Leo, UK) and subjected to XRD analysis.

Weight change and pH

Weight changes of SSS2 scaffold between two sampling points and changes in pH of SBF during acellular *in vitro* bioactivity study were recorded with an analytical balance of accuracy ±0.1 mg (Sartorius AG, Goettingen, Germany) and a pH meter with platinum pH electrode (Sension1, Hach, CO), respectively. The study was carried out with three replicates and the averages plotted.

Preparation of drug-loaded SSS2 scaffolds

SSS2 scaffolds were loaded with drug by vacuum infiltration method. A scaffold of known weight was immersed in drug solution and a vacuum of 9.67 ton/in^2 was applied. After 20 min, vacuum was released and the scaffolds were dried at room temperature. Selected

Table 1. Composition of scaffolds.

Scaffold code	Height of the scaffold (mm)	Concentration of the drug in loading solution (mg/ml)	Drug	Concentration of chitosan in coating solution (%W/V)	Dissolution medium
SSS2-1	3	25	Gatifloxacin	—	PBS
SSS2-2	6	25	Gatifloxacin	—	PBS
SSS2-3	12	25	Gatifloxacin	—	PBS
SSS2-4	12	12.5	Gatifloxacin	—	PBS
SSS2-5	12	6.25	Gatifloxacin	—	PBS
SSS2-6	12	25	Fluconazole	—	PBS
SSS2-7	12	25	Gatifloxacin	0.5	PBS
SSS2-8	12	25	Gatifloxacin	1.0	PBS
SSS2-9	12	25	Gatifloxacin	—	SBF

scaffold were coated with chitosan by immersing in a solution of chitosan of known concentration and subjecting a negative pressure for 5 min. The effect of different parameters as size of scaffold, initial concentration of the drug solution used for loading drug, types of drug used, and nature of coating used in the preparation of drug-loaded scaffolds on drug release were studied (Table 1).

Determination of drug loading in scaffolds

The amount of drug loaded in SSS2 scaffolds was calculated from the concentration difference of drug solution before and after the infiltration process (Zhu and Kaskel, 2009). The concentration of drug solution was determined by spectroscopic analysis using an UV spectrophotometer (PerkinElmer) at 287 and 261 nm for gatifloxacin and fluconazole, respectively.

Drug loading in scaffolds was determined from the following relationship

$$\text{Drug loading}(\%) = \frac{\left(\begin{array}{c}\text{amount of drug in}\\ \text{solution before loading}\end{array}\right) - \left(\begin{array}{c}\text{amount of drug in}\\ \text{solution after loading}\end{array}\right)}{\text{weight of the scaffold, g}} \times 100 \quad (1)$$

Invitro drug release studies

Drug-loaded SSS2 scaffolds were kept immersed in 5 ml of phosphate buffered saline (PBS) of pH 7.4 maintained at 37°C. Aliquots were removed at selected intervals from 24 h to 43 days and analysed by UV spectrophotometry. Study was continued until samples released drug above 1.2 μg/ml/day or 43 days, whichever was earlier. The set limit value was averaged per day by dividing the measured concentration with the number of days between sampling points. *In vitro* drug release studies for selected samples were also conducted in a similar way in SBF (pH 7.4).

Drug release kinetics

To study the drug release kinetics from SSS2 scaffolds, the *in vitro* drug release data of gatifloxacin in different dissolution media were fitted in five kinetic models. These models are zero order (Eq. 2), first order (Eq. 3), Higuchi (Eq. 4), Hixon-Crowell (Eq. 5), and Korsmeyer-Peppas (Eq. 6).

$$A_t = k_0 t \quad (2)$$

$$\log AR_t = k_1 t/2.303 \quad (3)$$

$$A_t = k_H t^{0.5} \quad (4)$$

$$(AR_t)^{1/3} = k_{HC} t \quad (5)$$

$$A_t/A_\infty = k_{KP} t^n \quad (6)$$

where A_t is the amount of drug released at time t; AR_t is the amount of unreleased drug at time t; $A\infty$ is the amount of drug released at time ∞; k_0, k_1, k_H, k_{HC}, and k_{KP} are the release constants for zero-order, first-order, Higuchi, Hixon-Crowell and Korsmeyer-Peppas models, respectively (Czarnobaj, 2008). In Eq. 6, n is the exponent indicative of the release mechanism.

Results

Characterization of SSS2 glass

From the FTIR spectrum (Figure 1), well-defined peaks characteristic of the stretching and bending vibrations of Si–O–Si bonds could be observed at around 469, 801, and 1041 cm^{-1}. In addition, two peaks at around 1415 and 1455 cm^{-1}, which are the characteristic of C–O group, were also observed. Emergence of this twin peaks could be due to the absorption of CO_2 from atmosphere even after thermal treatment (Radev et al., 2009) and hence, storage in dessicator is advised.

XRD patterns (Figure 2) indicated the amorphous nature of SSS2, which is desirable for *in vivo* applications (Nandi et al., 2009).

The *in vitro* acellular bioactivity of SSS2 glass was studied for a period of 1, 3, and 7 days. The FTIR spectra of SSS2 glass before (0 day) and after soaking in SBF for different periods (1, 3, and 7 days) are presented in Figure 3. Before soaking in SBF, the sample exhibited bending and stretching vibrations of Si–O–Si bonds at 469 and 1041 cm^{-1}. After soaking in SBF for 1 day, a bending vibrational bond of P–O was observed at 560 cm^{-1} indicating the growth of amorphous phosphate. Well-resolved peaks at 1428 and 1487 cm^{-1} assigned to C–O vibrational bands and at around 955, 1040, and 1220 cm^{-1} assigned to the P–O bonds could be observed indicating the formation of hydroxycarbonate apatite.

Characterization of porous scaffolds

Porous SSS2 scaffolds were fabricated by using naphthalene powder (as a porogen) of a particular size range (< 296 μm). However, porosity and porous nature of the scaffolds are strongly influenced by the properties of the used porogen and the sintering temperature of the scaffold (Soundrapandian et al., 2007; Kundu et al., 2010).

Table 2. Modeled invitro release kinetics.

					Peppas-Korsmeyer	
Formulations	Zero-order $R(2)$	First order $R(2)$	Higuchi $R(2)$	Hixon-Crowell $R(2)$	$R(2)$	n
SSS2-3	0.7072	0.9092	0.8662	0.7072	0.9492	0.34
SSS2-9	0.8272	0.9007	0.9474	0.8719	0.9888	0.29

Selecting naphthalene of a particular size range and subjecting the scaffolds to a fixed sintering procedure shall help to control pore size distribution between batches.

The bulk density of the SSS2 scaffolds was 1.016 g/ml. The true porosity and closed porosity were calculated to be 62.38% and 9.06%, respectively, with an apparent porosity of 53.32%. These values fall in the range generally agreed for rapid osteointegration (Hing, 2005).

The pores size distribution chart (Figure 4) of SSS2 scaffolds reports that the pores were predominantly in the range of 10–60 μm, indicating their suitability for drug delivery applications (Soundrapandian et al., 2007); as small pores facilitate high adsorption of drug and sustain the release better than the larger pores (Seeley et al., 2008).

The SEM images of SSS2 scaffolds subjected to acellular *in vitro* bioactivity study are presented in Figure 5. Although the scaffold structure was intact for the entire study period, the surface exhibited significant changes before (0d, SEM magnification: 1500×) and after soaking (1d, SEM magnification: 5000×; 3d SEM magnification: 60,000×; 7d SEM magnification: 5000×) in SBF. After soaking in SBF for 1 day, the surface appeared with apatite deposits, which required higher magnification for viewing. After 3 days, the deposits appeared as sporadic agglomerates and after 7 days, the deposits appeared as much bigger agglomerates of plates stacked on one another. Pores in micrometer range were also visible in the scaffold that was created either during fabrication or during deposition of apatites.

The XRD spectra of surface deposits on SSS2 scaffolds, following *in vitro* bioactivity studies is presented in Figure 6. After incubation in SBF, new peaks appeared at 26° and 32° of 2θ corresponding to hydroxyl carbonate apatite (HCAp) (JCPDS 04-0697). These results ensured the deposition of HCAp on scaffold.

Changes in weight of SSS2 scaffold on exposure to SBF and the change in pH of SBF are presented in

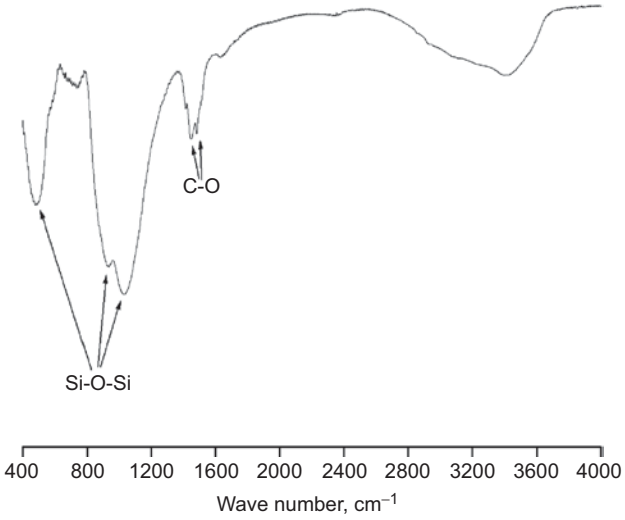

Figure 1. FTIR spectrum of SSS2 glass.

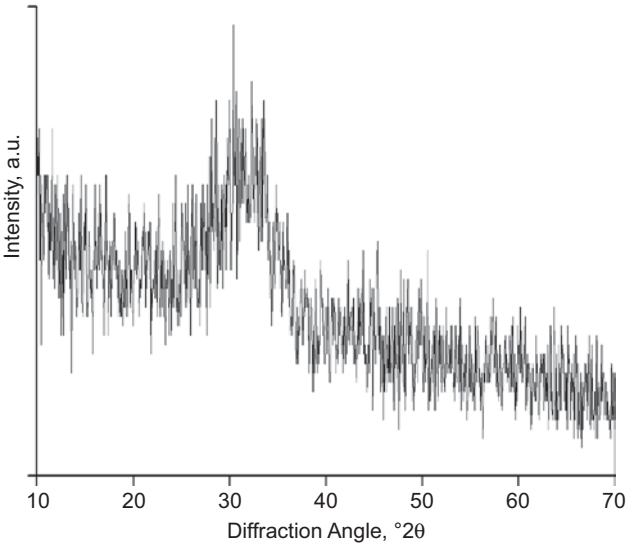

Figure 2. XRD spectrum of SSS2 glass.

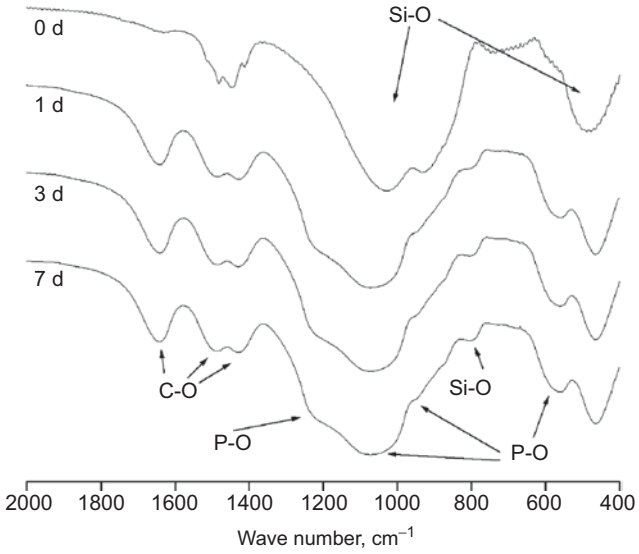

Figure 3. FTIR spectra of SSS2 glass subjected to acellular *in vitro* bioactivity study at various periods.

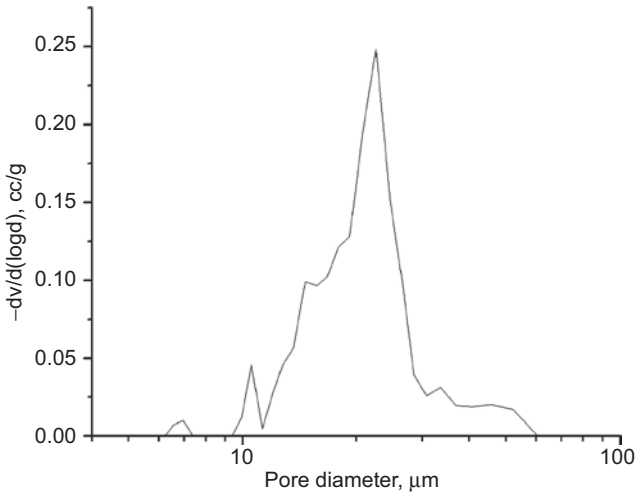

Figure 4. Pore size distribution in SSS2 scaffolds.

Figure 7. A total gain of about 7.13% W/W was observed at the end of a 4-week study period. The figure also shows an increase in pH from 7.4 to 8.85 in the first week. The high value was comparable with the result of other workers (Balamurugan et al., 2007). In the second week, it raised further to 9.03. However, for the rest of the study period, the pH was found to gradually decrease to a final value of 8.23.

Loading and *in vitro* drug release studies

The drug loading efficiency was calculated to be 3.9%. Figure 8 presents the release profiles of gatifloxacin from various sized scaffolds. Among the three scaffolds, SSS2-1 released the entire drug in 8 days. The formulations SSS2-2 and SSS2-3 were found to release the drug for the entire study period and above the set limit (1.2 μg/ml/day). Of the two formulations, SSS2-2 released more than 73% of the drug in the first day and about 90% in 2 days. Although, SSS2-3 released significant fraction of drug in the first day, it better sustained the release than SSS2-2. Hence, SSS2-3 scaffold was selected for further investigations.

Figure 9 presents the release profiles of gatifloxacin from scaffolds prepared by immersing in drug solutions of varying concentrations. Of the studied three formulations, SSS2-5 and SSS2-4, failed by releasing more than 97% and 95% of drug in 2 and 8 days, respectively. SSS2-3 was found better in sustaining the drug release, in this case too.

Figure 10 compares the release profiles of gatifloxacin (SSS2-3) and fluconazole (SSS2-6) from two identical SSS2 scaffolds. Both the drugs were released over the entire study period. Although 94.7% fluconazole was

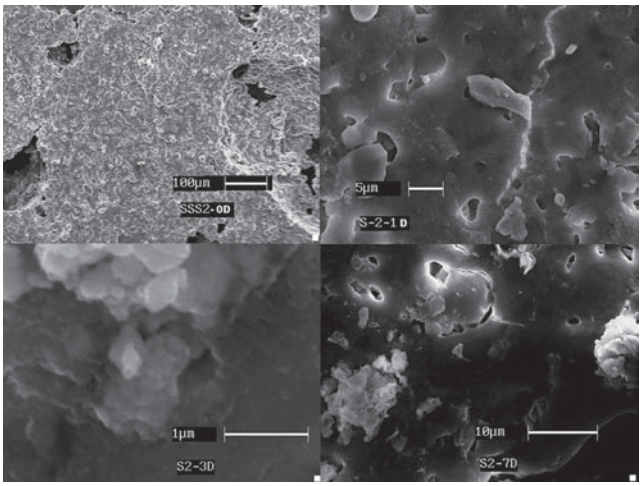

Figure 5. SEM images of SSS2 scaffold surface subjected to acellular *in vitro* bioactivity study at various periods. (0d - day zero, 1d - after 1 day, 3d - after 3 days, 7d - after 7 days) (SEM magnification was 1500× for 0d, 5000× for 1d, 60,000× for 3d, and 5000× for7d).

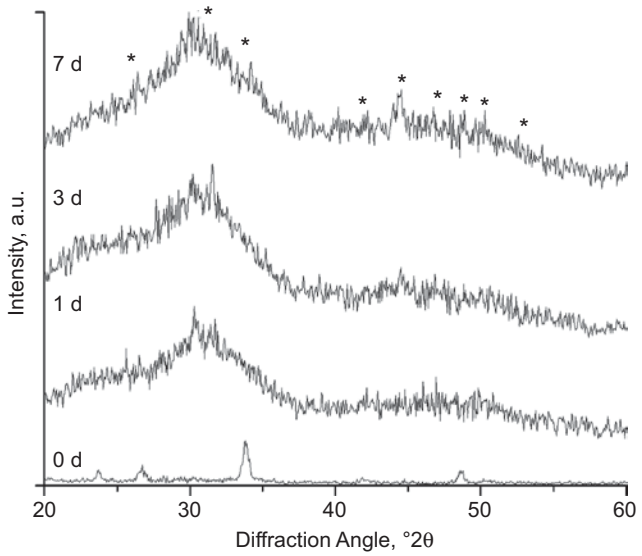

Figure 6. XRD spectra of SSS2 scaffold surface-deposit obtained at various periods of acellular *in vitro* bioactivity study (* indicates characteristic peaks of HCAp).

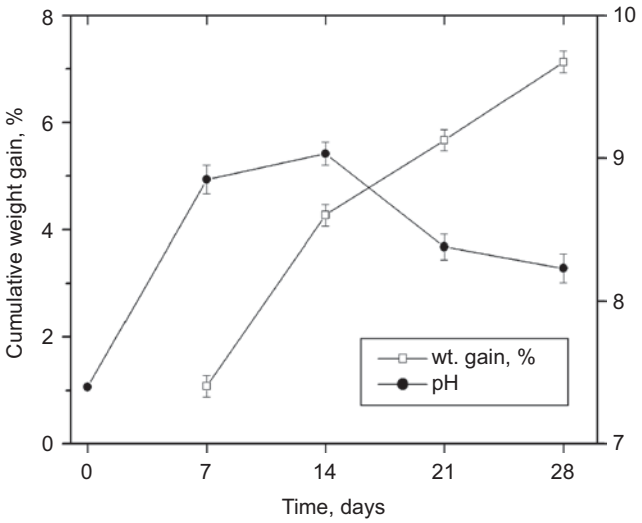

Figure 7. Pattern of change in the weight of SSS2 scaffolds and the change in pH of the SBF used in acellular *in vitro* bioactivity over the period of study.

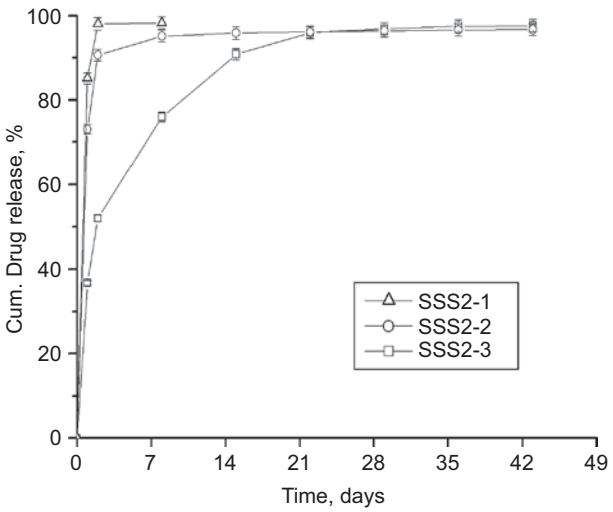

Figure 8. Effect of scaffold size on drug release.

released at the end of study period, 97.6% gatifloxacin was found to be released during the same period.

Figure 11 presents the release of gatifloxacin from uncoated (SSS2-3) and chitosan-coated (0.5% - SSS2-7; 1% - SSS2-8) scaffolds. Although coating of the scaffolds reduced the burst release significantly, SSS2-8 formulation that was coated with higher concentration of chitosan (1% W/V) failed to maintain the set limit (1.2 µg/ml/day) in the last week. On the other hand, SSS2-7, coated with 0.5% chitosan was able both to reduce the burst release and to maintain the release concentration above the set limit for the entire study period.

Figure 12 presents the effect of dissolution medium on drug release using SSS2-3. The difference in release profiles started from the first 24 h, itself. Drug release in SBF was lower than that in PBS and the difference got wider with progress in time. Although SSS2-3 released about 36.8% of drug in the first 24 h, SSS2-9 released about 28.1%, The maximum concentration of drug released per day from SSS2-3 and SSS2-9 were 1915.6 and 1459.7 µg/mL, respectively, and the minimum concentration 1.2 and 2.1 µg/ml in PBS and SBF, respectively. At the end of study period, the amount of drug released in SBF was about 22% less than that in PBS, although the release of the drug was above the set concentration limit.

Comparing the results of various models (Table 2) better correlation was observed with Korsmeyer-Peppas model by fickian diffusion.

Discussion

Application of biomaterials for bone substitution as well as for drug release locally in the bone is interestingly becoming more popular (Soundrapandian et al., 2009). Collagen, polyurethanes in the form of implants and sponges were able to deliver antibiotics but the release complete in a maximum of 5 days (Wachol-Drewek et al., 1996, Schierholz et al., 1997). Success has been reported with dextran/gelatin in making available drugs such as basic fibroblast growth factor in active form and in higher concentrations, locally (Gu et al., 2009). Recently,

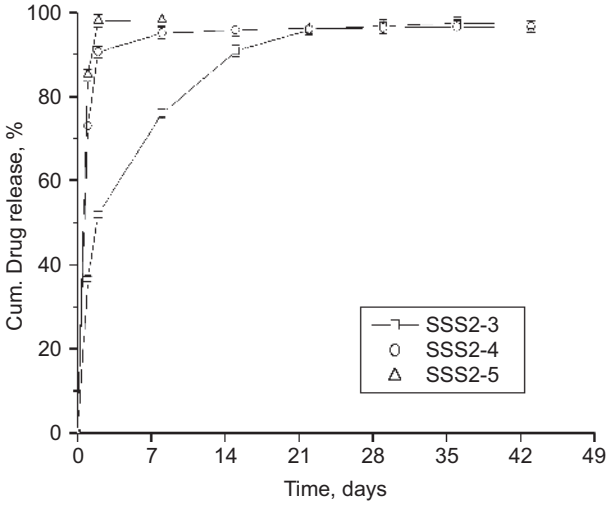

Figure 9. Effect of concentration of drug in loading solution on drug release.

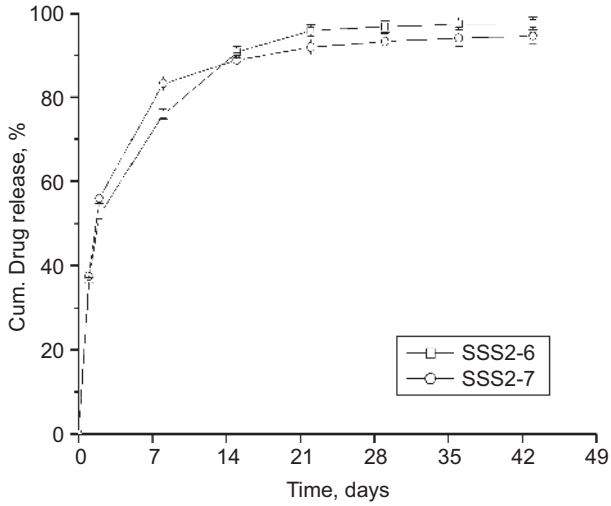

Figure 10. Release profiles of two different drugs.

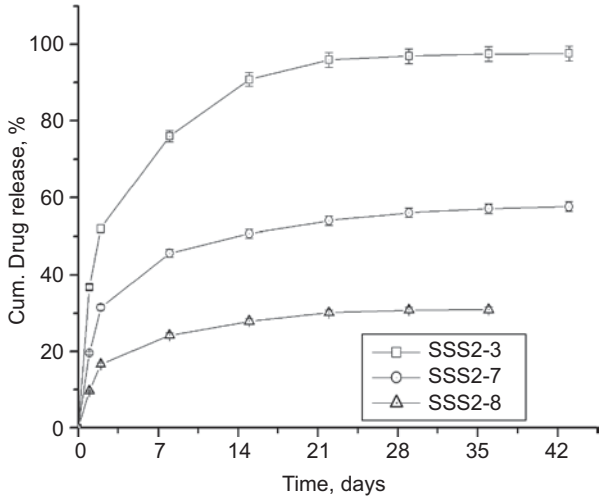

Figure 11. ffect of coating and concentration of polymer solution on drug release.

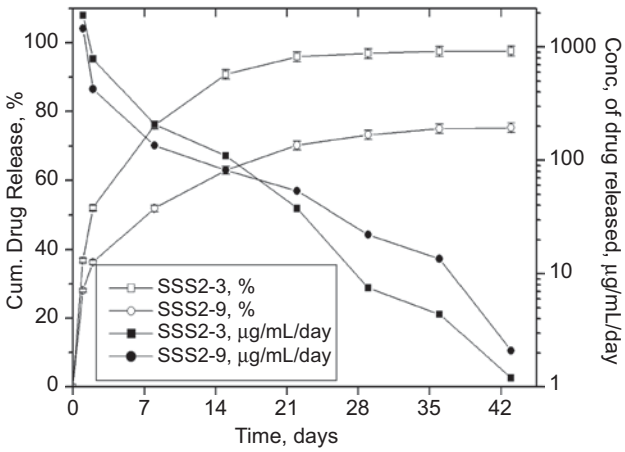

Figure 12. Effect of dissolution medium on drug release.

Chevalier et al. (2009) compared the effects of processing implantable calcium phosphate granules.

Bioactive glasses are fascinating materials as they are capable of both bonding with bone and sustaining the release of drugs (Zhao et al., 2008). Silica-based quaternary glass systems containing SiO_2, Na_2O, CaO, and P_2O_5 in certain compositions are known to be bioactive. Bioactivity of the glasses is expected to be high with chemical compositions of $SiO_2 < 60$ mol%, high Na_2O and CaO contents, and a high CaO/P_2O_5 ratio (Agathopoulos et al., 2006; Bang et al., 2008). Addition of intermediate or modifying oxides (e.g., zinc oxide) to the base composition can also modify bioactivity. Further, zinc is considered to activate bone formation, inhibit bone resorption, and improve mechanical properties (Aina et al., 2007). In this study, a new composition of bioactive glass (SSS2) containing zinc has been developed to study its suitability as a porous scaffold for local drug delivery in osteomyelitis.

Osteomyelitis demands high concentration of antimicrobials in the pathological site for several weeks. Conventional delivery systems often fail to maintain drugs at high concentrations in the local area due to various reasons. From the drug point of view, factors such as molecular size, protein binding, fat solubility, and partition coefficient, and physiological factors such as organ perfusion, blood flow, and rate of flow seriously restrict drug availability at the expected site (Hughes and Anderson, 1985; Soundrapandian et al., 2007). Local drug delivery systems bypass all the aforementioned restrictions and provide high local concentrations of drugs for prolonged periods in addition to low or nil systemic drug concentrations that could produce serious side effects.

Bioactivity is the phenomena of eliciting a biological response in the material–biological site interface resulting in the formation of bonding between them, following a series of biophysical and biochemical reactions at the interface. Bioactivity can be tested *in vitro* by the ability of the material to deposit apatites on its surface. Development of a layer consisting carbonate-containing hydroxylapatite (HCAp) on the surface of SSS2 indicates its ability to bond with bone (Kontonasaki et al., 2002). Unlike polymers, bioactives favor apatite deposition and hence over a period, weight gain of these scaffolds is obvious. Bioactives do experience weight loss. However, this phase occurs significantly within the first week. It could be observed from Figure 7 that the weight gain in the initial first week when compared with the following weeks was low, possibly due to a faster leaching of ions such as Si^+, Ca^{2+}, Na^+, and PO_4^{3-} from the scaffold than the deposition of apatites. From the second week to the fourth week, it was almost linear. Similar results of weight gain of bioactives could be found only in case where degradation (as usually mentioned) studies have been conducted for weeks together as with bioactive tricalcium phosphate (Seeley et al., 2008). The increase in pH was possibly due to the leaching of ions such as $Ca^{2+}$and Na^+ from the scaffold. However, it is advantageous, as it creates a favorable atmosphere for apatite deposition. The steep increase in weight at the end of second week and the pH at the respective time substantiate the statement.

Gatifloxacin, a relatively recent fluoroquinolone and a better choice than nafcillin in the treatment of experimental osteomyelitis (Shirtliff et al., 2002) was selected as the model drug. Selection of drugs for a formulation is largely dictated by their physicochemical and pharmacokinetic properties in conventional drug delivery systems. In contrast, local drug delivery systems offer a unique advantage of overlooking such issues thereby offering a broader scope for drug selection. Optimal drug delivery in conventional drug delivery systems is limited due to various pharmacokinetic and other associated parameters such as molecular size and plasma protein binding. In localized drug delivery systems, these issues are inconsequential, because the drugs circumvent such biological barriers by being released in the site of action. Further, the methodology used herein is purported to be seldom influenced by the physicochemical properties of the incorporated drug, because the only processing technique postdrug incorporation involves drying at room temperature.

For *in vitro* drug release, cutoff limit of drug was set at 1.2 μg/ml/day, 10 times the minimum inhibitory concentration (MIC) of *Staphylococcus aureus* (NCCLS, 2002). The intention was to deliver drug for the treatment of osteomyelitis. Based on microorganism, drug release at concentrations above MIC could be considered acceptable. But in a condition when organisms are often capable of evading host defense mechanisms and even drugs at MIC and resulting in the formation of biofilms, it is reasonable to set limit not just at MIC but at biofilm eradication concentration. Biofilms are the main cause for relapse of infection and generally demand more than 10-folds the MIC to kill planktonic cells (Soundrapandian et al., 2007).

In vitro drug release studies for the first three formulations (SSS2-1, SSS2-2, and SSS2-3) indicate the influence of size of scaffolds. Increasing the length of the scaffold increases the path length of the drug to travel manifold before it is released into the dissolution medium. With the size of drug molecules in nanometers, the effect of increase in length of scaffold by even 1 mm could be highly influential. With the size of the scaffold optimized with the results of Figure 8A, the next intention was to reduce the concentration of drug in the initial drug loading solution, if possible. Decrease in concentration of drug in the loading solution resulted in lower entrapment of drug within the SSS2 scaffold (data not presented here). Loading of scaffolds from dilute drug solution probably left the pores of the channels inside the scaffolds less congested, causing less resistance to the drug molecules during diffusion and resulted in faster drug release.

Fluconazole was selected as the second model drug as it represents the antifungal drugs. Suitability of the SSS2 scaffolds in sustaining the release of fluconazole broadens the spectrum of application in osteomyelitis

as fungal osteomyelitis is now not an uncommon disease. Although SSS2 scaffolds were capable of releasing gatifloxacin and fluconazole for more than 6 weeks in a rate above the set limit, a considerable part of the drugs released in the first day. Bioactive scaffolds act more like a reservoir with drug molecules existing in the scaffolds in four different states (Xia and Chang, 2006). A considerable fraction of drug molecules is attached to the exterior surface of the scaffold while the major fraction gets trapped in the pore channels where drug molecules exist with or without bonding to the pore wall surface. Both the drugs were loaded in SSS2 scaffolds by immersing the scaffolds in drug solution and applying negative pressure. This results in the attachment of drug molecules in the pore channels and on the scaffold surface. In addition, both the drug molecules contain highly electronegative atoms/groups such as F, N, and OH, which could form hydrogen bonds with Si–OH and P–OH groups in the scaffold or pore wall surface. Hence, it is obvious that the drug molecules adhered on the surface get released faster contributing much of the burst release in the first day, whereas those inside the pore channels are released in a sustained fashion, as the drug has to diffuse through the channels.

Coating the scaffolds could significantly reduce burst release and coating bioceramic scaffolds with polymer results in the formation of organic–inorganic composite scaffolds. Chitosan was selected as it is a natural polysaccharide with excellent biocompatibility, nontoxicity, biodegradability, and bioresorbability (Rossi et al., 2008; Cekic et al., 2009; Kählig et al., 2009; Lin et al., 2009; Ubaidulla et al., 2009). Properties such as antibacterial, antifungal, haemostatic, and wound healing (Kong et al., 2008; Park et al., 2008; Baldrick, 2010) along with their various biological activities make it an interesting polymer for selection. The effect of coating of SSS2 scaffolds with chitosan on drug release was compared with SSS2-3. Although, coating could be applied to control drug release, high concentration of polymers may restrict the release of drugs to an extent that the concentration of release does not meet the set limit, as with SSS2-8 in the last week of study (Figure 8D). Presence of polymer coat on bioceramics may also affect bioactivity, by delaying the process of apatite deposition (Soundrapandian et al., 2009).

Drug release studies are generally conducted with PBS as dissolution medium (Soundrapandian et al., 2007, 2009). However, SSS2 glass/scaffold reacts with SBF and form apatites on the surface, which could alter drug release. Comparative drug release studies in PBS and SBF (Figure 8E) indicate that the same scaffold could deliver drugs successfully for a few weeks more in SBF, proving the release of drug from the scaffolds also influenced by the formation of apatites. Burst release phenomena were observed in both the dissolution media. However, both the burst release quantities were far lower than the release reported earlier for the same drug from nonporous monolithic-polymer-controlled systems (El-Kamel and Baddour, 2007).

Finally, theoretical analysis and models of drug release used to describe release from polymers can also be applied with bioceramic carrier systems (Melville et al., 2008). Drug release from SSS2 scaffolds in both the dissolution media (PBS and SBF) were found to fit with Peppas-Korsmeyer model. With "n" values less than 0.45, release from these cylindrical samples could be understood to follow Fickian diffusion. Although erosion of SSS2 scaffolds happen in both the dissolution media, their influence in drug release seemed to be the least depending on the R^2 values of Higuchi, Hixon-Crowell, and Peppas-Korsmeyer models.

Conclusion

Local drug delivery system for the treatment of osteomyelitis was prepared based on a new composition of zinc-containing glass (SSS2). Both the SSS2 glass and scaffolds were bioactive. The SSS2 scaffolds were able to sustain the release of drugs successfully for 6 weeks irrespective of the dissolution medium while the drug followed first-order kinetics based on diffusion mechanism.

The results indicated that the newly developed bioactive scaffold (SSS2) has a potential for use in osteomyelitis treatment.

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Declaration of interest

The authors declared no conflict of interest.

References

Agathopoulos S, Tulyaganov DU, Ventura JMG, Kannan S, Saranti A, Karakassides MA, Ferreira JMF. (2006). Structural analysis and devitrification of glasses based on the CaO-MgO-SiO2 system with B2O3, Na_2O, CaF_2 and P_2O_5 additives. J Non-Cryst Solids, 352:322–328.

Aina V, Perardi A, Bergandi L, Malavasi G, Menabue L, Morterra C et al. (2007). Cytotoxicity of zinc-containing bioactive glasses in contact with human osteoblasts. Chem Biol Interact, 167:207–218.

Andrade AL, Souza DM, Vasconcellos WA, Ferreira RV, Domingues RZ. (2009). Tetracycline and/or hydrocortisone incorporation and release by bioactive glasses compounds. J NonCryst Solids, 355:811–816.

Balamurugan A, Balossier G, Michel J, Kannan S, Benhayoune H, Rebelo AH et al. (2007). Sol gel derived SiO(2)-CaO-MgO-P(2)O(5) bioglass system–preparation and *in vitro* characterization. J Biomed Mater Res Part B Appl Biomater, 83:546–553.

Baldrick P. (2010). The safety of chitosan as a pharmaceutical excipient. Regul Toxicol Pharmacol, 56:290–299.

Bang H-G, Kim S-J, Park S-Y. (2008). Biocompatibility and the physical properties of bio-glass ceramics in the Na_2O-CaO-SiO_2-P_2O_5 system with CaF_2 and MgF_2 additives. J Ceram Process Res, 9:588–590.

Cekic ND, Milic JR, Savic SD, Savic MM, Jovic Z, Daniels R. (2009). Influence of the preparation procedure and chitosan type on physicochemical properties and release behavior of alginate-chitosan microparticles. Drug Dev Ind Pharm, 35:1092–1102.

Chevalier E, Viana M, Cazalbou S, Chulia D. (2009). Comparison of low-shear and high-shear granulation processes: effect on implantable calcium phosphate granule properties. Drug Dev Ind Pharm, 35:1255–1263.

Czarnobaj K. (2008). Preparation and characterization of silica xerogels as carriers for drugs. Drug Deliv, 15:485–492.

El-Ghannam A, Ahmed K, Omran M. (2005). Nanoporous delivery system to treat osteomyelitis and regenerate bone: gentamicin release kinetics and bactericidal effect. J Biomed Mater Res Part B Appl Biomater, 73:277–284.

El-Kamel AH, Baddour MM. (2007). Gatifloxacin biodegradable implant for treatment of experimental osteomyelitis: *in vitro* and *in vivo* evaluation. Drug Deliv, 14:349–356.

Gu C, Zheng R, Yang Z, Wen A, Wu H, Zhang H et al. (2009). Novel glycidyl methacrylated dextran/gelatin nanoparticles loaded with basic fibroblast growth factor: formulation and characteristics. Drug Dev Ind Pharm, 35:1419–1429.

Hing KA. (2005). Bioceramic bone graft substitutes: influence of porosity and chemistry. Int J Appl Ceram Technol, 2:184–199.

Hughes SP, Anderson FM. (1985). Penetration of antibiotics into bone. J Antimicrob Chemother, 15:517–519.

Itokazu M, Ohno T, Tanemori T, Wada E, Kato N, Watanabe K. (1997). Antibiotic-loaded hydroxyapatite blocks in the treatment of experimental osteomyelitis in rats. J Med Microbiol, 46:779–783.

Kählig H, Hasanovic A, Biruss B, Höller S, Grim J, Valenta C. (2009). Chitosan-glycolic acid: a possible matrix for progesterone delivery into skin. Drug Dev Ind Pharm, 35:997–1002.

Kong M, Chen XG, Liu CS, Liu CG, Meng XH, Yu le J. (2008). Antibacterial mechanism of chitosan microspheres in a solid dispersing system against E. coli. Colloids Surf B Biointerfaces, 65:197–202.

Kontonasaki E, Zorba T, Papadopoulou L, Pavlidou E, Chatzistavrou X, Paraskevopoulos K, Koidis P. (2002). Hydroxy carbonate apatite formation on particulate bioglass *in vitro* as a function of time. Cryst Res Technol, 37:1165–1171.

Kundu B, Soundrapandian C, Nandi SK, Mukherjee P, Dandapat N, Roy S et al. (2010). Development of new localized drug delivery system based on ceftriaxone-sulbactam composite drug impregnated porous hydroxyapatite: a systematic approach for *in vitro* and *in vivo* animal trial. Pharm Res, 27:1659–1676.

Lin A, Chen J, Liu Y, Deng S, Wu Z, Huang Y, Ping Q. (2009). Preparation and evaluation of N-caproyl chitosan nanoparticles surface modified with glycyrrhizin for hepatocyte targeting. Drug Dev Ind Pharm, 35:1348–1355.

Melville AJ, Rodríguez-Lorenzo LM, Forsythe JS. (2008). Effects of calcination temperature on the drug delivery behaviour of Ibuprofen from hydroxyapatite powders. J Mater Sci Mater Med, 19:1187–1195.

Nandi SK, Kundu B, Mukherjee P, Mandal TK, Datta S, De DK, Basu D. (2009). *In vitro* and *in vivo* release of cefuroxime axetil from bioactive glass as an implantable delivery system in experimental osteomyelitis. Ceram Int, 35:3207–3216.

NCCLS. (2002). Performance standards for antimicrobial susceptibility testing. NCCLS, Wayne, Pa.

Ouédraogo M, Semdé R, Somé IT, Traoré-Ouédraogo R, Guissou IP, Henschel V et al. (2008). Monoolein-water liquid crystalline gels of gentamicin as bioresorbable implants for the local treatment of chronic osteomyelitis: *in vitro* characterization. Drug Dev Ind Pharm, 34:753–760.

Park Y, Kim MH, Park SC, Cheong H, Jang MK, Nah JW et al. (2008). Investigation of the antifungal activity and mechanism of action of LMWS-chitosan. J Microbiol Biotechnol, 18:1729–1734.

Radev L, Hristov V, Samuneva B, Ivanova D. (2009). Organic/inorganic bioactive materials Part II: *in vitro* bioactivity of collagen-calcium phosphate silicate/wollastonite hybrids. Cent Eur J Chem, 7:711–720.

Rossi S, Marciello M, Sandri G, Bonferoni MC, Ferrari F, Caramella C. (2008). Chitosan ascorbate: a chitosan salt with improved penetration enhancement properties. Pharm Dev Technol, 13:513–521.

Schierholz JM, Steinhauser H, Rump AF, Berkels R, Pulverer G. (1997). Controlled release of antibiotics from biomedical polyurethanes: morphological and structural features. Biomaterials, 18:839–844.

Seeley Z, Bandyopadhyay A, Bose S. (2008). Tricalcium phosphate based resorbable ceramics: Influence of NaF and CaO addition. Mat Sci Eng C, 28:11–17.

Shirtliff ME, Calhoun JH, Mader JT. (2002). Gatifloxacin efficacy in treatment of experimental methicillin-sensitive Staphylococcus aureus-induced osteomyelitis in rabbits. Antimicrob Agents Chemother, 46:231–233.

Soundrapandian C, Datta S, Sa B. (2007). Drug-eluting implants for osteomyelitis. Crit Rev Ther Drug Carrier Syst, 24:493–545.

Soundrapandian C, Sa B, Datta S. (2009). Organic-inorganic composites for bone drug delivery. AAPS Pharmscitech, 10:1158–1171.

Tian Y, Li L, Gao X, Deng J, Stephens D, Robinson D et al. (2002). The effect of storage temperatures on the *in vitro* properties of a polyanhydride implant containing gentamicin. Drug Dev Ind Pharm, 28:897–903.

Ubaidulla U, Khar RK, Ahmad FJ, Tripathi P. (2009). Optimization of chitosan succinate and chitosan phthalate microspheres for oral delivery of insulin using response surface methodology. Pharm Dev Technol, 14:96–105.

Wachol-Drewek Z, Pfeiffer M, Scholl E. (1996). Comparative investigation of drug delivery of collagen implants saturated in antibiotic solutions and a sponge containing gentamicin. Biomaterials, 17:1733–1738.

Xia W, Chang J. (2006). Well-ordered mesoporous bioactive glasses (MBG): a promising bioactive drug delivery system. J Control Release, 110:522–530.

Zhao L, Yan X, Zhou X, Zhou L, Wang H, Tang J, Yu C. (2008). Mesoporous bioactive glasses for controlled drug release. Micropor Mesopor Mat, 109:210–215.

Zhu Y, Kaskel S. (2009). Comparison of the *in vitro* bioactivity and drug release property of mesoporous bioactive glasses (MBGs) and bioactive glasses (BGs) scaffolds. Micropor Mesopor Mat, 118:176–182.