AAPS PharmSciTech, Vol. 10, No. 4, December 2009 (© 2009)
DOI: 10.1208/s12249-009-9308-0

Mini-Review

Organic–Inorganic Composites for Bone Drug Delivery

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Received 3 November 2008; accepted 18 September 2009; published online 20 October 2009

Abstract. This review paper attempts to provide an overview in the fabrication and application of organic–inorganic based composites in the field of local drug delivery for bone. The concept of local drug delivery exists for a few decades. However, local drug delivery in bone and specially application of composites for delivery of drugs to bone is an area for potential research interest in the recent time. The advantages attained by an organic–inorganic composite when compared to its individual components include their ability to release drug, adopting to the natural environment and supporting local area until complete bone regeneration, which make them carriers of interest for local drug delivery for bone.

KEY WORDS: biomaterials; carrier systems; composite; local delivery; organic–inorganic.

INTRODUCTION

Steady increase in geriatric population, changes in lifestyle, and adverse effects of modernization have contributed to steep increase in the number of orthopedic patients in recent years. Over 1.5 million osteoporotic fractures are reported annually in the USA alone, costing approximately $15 billion each year (1), and an estimated 60 million people will be disabled or injured in developing countries in the next 10 years (2). About 50,000 hip replacements (and as many knees) are performed every year in the UK, and 193,000 are performed in the USA for osteoarthritis (3). Infective complications occur in 2–6% of patients following prosthetic joint replacement of the hip (4) and 0.7–9% following knee arthroplasty (5–7). In parallel, also the number of medications to treat and even prevent these diseases has expanded significantly in recent years (8). However, success in therapy has not been overwhelming as with other disease conditions. Key issues for failure include the inability to maximize drug access to bone and maintain optimum drug concentration for prolonged periods of time. To alleviate the drawbacks of conventional therapy, local drug delivery to bone had been tried by modern medicine from the 1970s (9). However, it is from the year 2000 that research on local delivery of drug to bone has gained considerable attention (Table I). Note that the numbers of publications in the last 3 years are double and decuple the number published in earlier decades.

Bone substitutes as acrylic polymers, biodegradable polymers, and ceramics are popular choices for carriers to bone drug delivery, and reviews based on them do exist (10–12). However, expectations of these implants to exhibit bioactivity and favor tissue regeneration in addition to controlled drug delivery could hardly be achieved with either class of bone substitutes. On the other hand, implants based on composites (organic–inorganic) of bone substitutes are able to meet the expectations (13–15). Widespread application of composites exists in the fields as construction engineering, polymeric research, *etc*. However, application of composites for the fabrication of drug delivery devices that can be placed in the bone is still in its infancy. This review is based only on those articles that have applied organic (polymer)–inorganic [inorganic chemicals, bioactive glass (BG), and bioceramics] composites for bone drug delivery. The purpose of this article is to provide an overview about the role of organic–inorganic composites for local drug delivery in bone, their fabrication, and important properties to be considered on development of the same. A review based on organic–inorganic composites for drug delivery to bone locally does not exist, at least to our knowledge. However, this article is not encyclopedic; rather, select examples have been chosen to summarize the progress.

NEED FOR COMPOSITES

Most natural materials are composites made up of both inorganic and organic components organized in complex structures. Bone is a composite matrix of collagen (organic) strengthened with hydroxyapatite (Hap) (inorganic). Within and around the composite matrix are located the bone cells, namely, osteoblasts, osteocytes, osteoclasts, and osteoprogenitor cells.

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ABBREVIATIONS: BG, Bioactive glass; BMP, Bone morphogenetic protein; CS, Calcium sulfate; DMC, Dimethyl carbonate; Hap, Hydroxyapatite; PCL, Polycaprolactone; PDLLA, Poly (D,L-lactide); PHBV, Polyhydroxybutyrate-*co*-hydroxyvalerate; PLGA, Polylactide-*co*-glycolide; PMMA, Polymethylmethacrylate; rh, Recombinant human; SBF, Simulated body fluid; TCP, Tricalcium phosphate; TGF, Transforming growth factor.

Table I. A List Indicating the Number of Publications on Local Delivery to Bone as Indexed in PubMed

Years	Number of publications
2006–2008	63
2000–2005	73
1990–1999	34
1980–1989	06

Various materials from stable or degradable polymers to inorganics, including glass and ceramics, have been notably tried for local drug delivery in bone. The carrier materials selected for drug delivery in bone are expected to be affordable, exhibit predictable release characteristics, mechanically and biologically compatible with local bone tissue, and possibly bioactive, bioresorbable, and osteoconductive/osteoinductive. While bioactive materials (e.g., BG) bonds physically and chemically to bone, a bioresorbable material [e.g., calcium sulfate (CS)] is resorbed slowly, ideally substituted by new bone formation. In addition, while osteoconductive materials [e.g., tricalcium phosphate (TCP)] are suitable for bone cells to attach, grow, multiply, and spread, osteoinductive materials (e.g. Hap) are capable of stimulating primitive stem cells or immature bone cells to grow and mature. Although each carrier material shall exhibit remarkable advantage over others, non-degradability and compulsion for a second surgery on healing site to remove the stable polymers, high cost, impaired osteoconduction, and adverse tissue response of biodegradable polymers, unsuitability of glass for load bearing regions of bone, and lack of ductility and poor degradability with ceramics are significant limitations to overcome (16–19). Hence, the option for an organic–inorganic composite material would be more rational than individual categories of carrier materials. Comprised of multiple phases mixed to provide better performance, composites are expected to provide desired mechanical stability, improve tissue integration, and retard drug release better. Table II presents a representative list of studies published based on organic–inorganic composites as drug delivery systems for bone. Figure 1 depicts some possible combinations in the formation of organic–inorganic composites for bone drug delivery.

BONE, DRUGS, AND LOCAL DELIVERY

Bones are rigid organs serving various vital functions in the body. In addition to providing shape, aiding in movement, and providing protection for vital inner organs, they are the production house of blood cells, storage house of fat, mineral, and growth factors, and play considerable role in detoxification and acid–base balance of blood. However, bone in general is a poorly perfused organ. During pathological conditions, the blood supply in the local area gets further hampered, making the availability of drug rather questionable. Although parenteral route is expected to provide better bioavailability and avoid the possibility of hepatic first pass metabolism as compared with oral route, bone being a poorly perfused organ is the major reason for experiencing poor supply of drugs at the site of treatment. Hence, the need to administer drugs at high doses and for prolonged periods, in conventional therapy, would appear logical. For example ceftriaxone should be administered at 2 g a day by i.v. for 4–6 weeks in the treatment of bone infections (41) and bisphosphonates as Risedronate at 5 mg per day orally at least for 6 months in case of osteoporosis (42). From the drug point, delivering large amounts of drugs to the body may display an increase in bioavailability but could result with exorbitant irrational quantities of drug getting eliminated from the body with potential increase in undesired serious side effects as nephrotoxicity and hepatotoxicity and escalated treatment cost, as with antimicrobials (43). In certain cases of drugs as growth factors, even a high dose of growth factors injected directly as an aqueous solution might struggle to maintain the desired biologic effects *in vivo* because of *in vivo* metabolism and enzymatic digestion resulting in short half-life of the drug in the body (28,44). Local delivery of drugs offer various possibilities for avoiding serious side effects, avoiding infusions, decreased hospitalization, reduced medical expenses, release drug in a sustained fashion, maintain high drug concentrations locally, reduce presence of drug in systemic blood circulation, maintain drug stability for a longer period, *etc*. Therefore, it becomes indispensable to seek local release of drugs for improved efficacy and faster healing.

Antibiotics

Of the various categories of drugs researched for local delivery in bone, antibiotics occupy a major section. Normal bone is highly resistant to infection, which can only develop as a result of trauma, very large inocula, or due to the presence of foreign bodies. Irrespective of the advancement in making surgeries and prosthesis, available sterile, and achieving aseptic conditions in operation theatres, infection associated with major surgeries are still unavoidable. Due to their application for prophylaxis and therapeutics antibiotics need to be applied to bone in every case of surgery, in addition to cases of bone infections. When the microbial load has crossed a critical density, they form biofilms that are quite hard for antibiotics to penetrate, often resulting in relapse of infection (45). Very high concentrations of antibiotics are required to eradicate them, which could hardly be attained by conventional routes of delivery without serious side effects.

Antibiotics in general are hydrophilic drugs, hardly exhibit stability problems (except a few as cephalosporins) making them suitable to load with any kind of composite. Release of antibiotic shall depend on various factors. However, insufficient release of antibiotics on the basis of time and concentration could lead to development of resistant strains and growth of microorganisms on the surface of the scaffolds.

Growth Factors

Although bone has the capability of self-regenerating or remodeling to a certain extent, the process is rather slow. Before operating bone, a question more frequented would be how fast it would be healed. It is estimated that delayed or impaired healing will occur in 5–10% of the 5.6 million fractures that occur annually in the USA, and up to 10% of all fractures will require additional surgical procedures for impaired healing (46). Delayed bone healing leads to socio-

Table II. Composites Studied for Drug Delivery in Bone

Composite					
Inorganic	Organic	Delivery system form	Drug	Type of study	References
β-TCP	PCL	Disks	Gatifloxacin hydrate	*In vivo*	(20)
CS	Versa Bond™	Rods	Teicoplanin	*In vitro*	(21)
TCP	PCL	Blocks	rhBMP-2	*In vitro*	(22)
Biphasic calcium phosphate	PDLLA	Discs	Vancomycin (with poly ethylene glycol)	*In vitro*	(23)
Tetra calcium phosphate – dicalcium phosphate anhydride	PLGA 50:50	Cylinders	Gentamicin crobefate (in PLGA microspheres)	*In vitro*	(24)
Calcibon™	PLGA 53:47	Discs	rhBMP-2 (in PLGA microspheres)	*In vivo*	(25)
BG	Poly(L-lactide-co-glycolide) 80:20	Rods	Ciprofloxacin	*In vitro* and *In vivo*	(26)
Wollastonite	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)	Microspheres	Gentamicin	*In vitro*	(27)
Phosphate glass	PCL	Blocks	Vancomycin	*In vitro*	(28)
β-TCP	Chitosan	Microgranules	rhTGF-β1	*In vitro*	(14)
Hap	PCL	Coat	Tetracycline hydrochloride	*In vitro*	(29)
Invert calcium phosphate glass – β-TCP	Chitosan	Blocks	Gentamicin sulfate	*In vitro*	(30)
Coralline Hap	Gelatin	Microspheres	Gentamicin	*In vitro*	(31)
BG	PMMA	Bars	Gentamicin sulfate	*In vitro*	(32)
BG	PLLA–PMMA	Bars	Gentamicn sulfate	*In vitro*	(33)
Hap	Anionic collagen gel	Discs	Norfloxacin, Ciprofloxacin, Gentamicin	*In vitro*	(34)
Titanium	PCL	Discs	Ampicillin sodium	*In vitro*	(35)
Zirconium	PCL	Discs	Ampicillin sodium	*In vitro*	(36)
Hap	Alginate	Beads	Paclitaxel	*In vivo*	(37)
Hap	Gelatin	Coat	Ibuprofen	*In vitro*	(38)
Silicas	PMMA	Film	Aspirin	*In vitro*	(39)
Tetra calcium phosphate and Dicalcium phosphate	Collagen	–	Estradiol	*In vitro* & *Invivo*	(40)

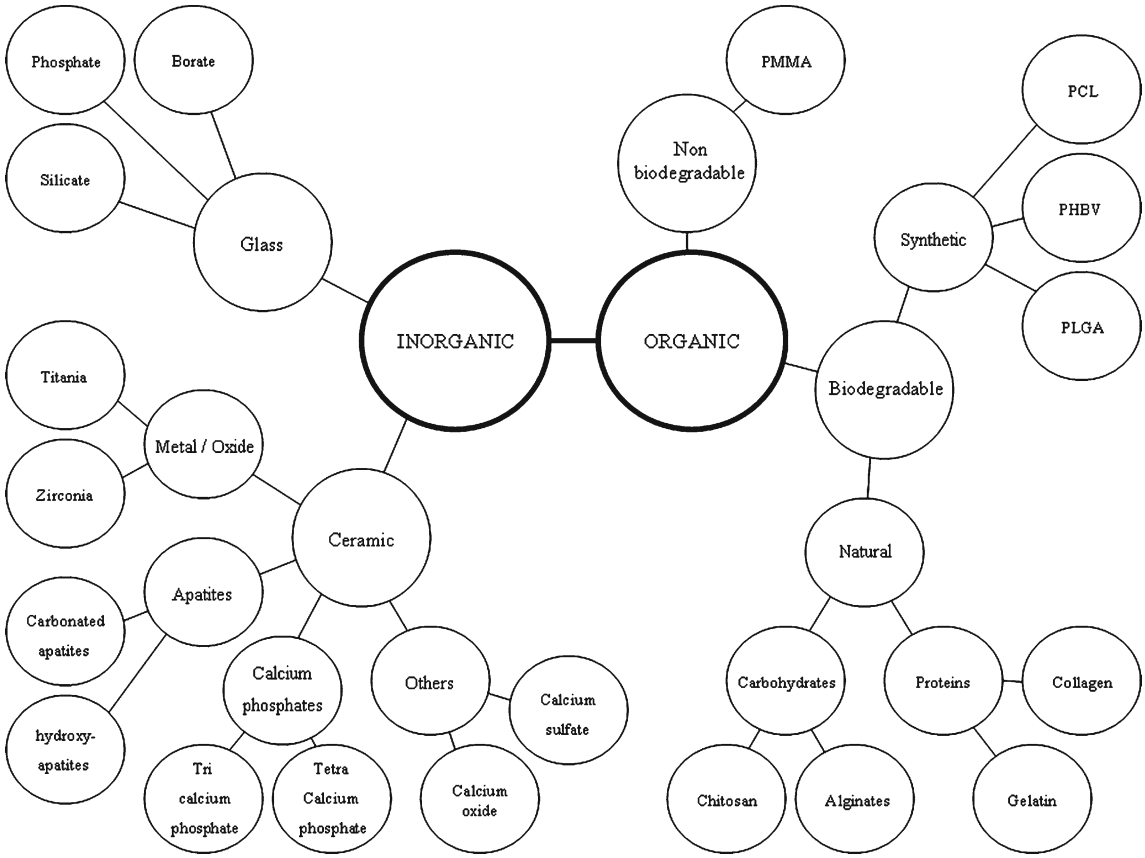


Fig. 1. Some possible combinations in the formation of organic–inorganic composite

economic costs of up to 14.7 billion Euros per year in Europe alone (47). Bone healing is a slow and complex physiological process initiated and controlled by growth factors and the healing potential influenced by a variety of biochemical, biomechanical, cellular, hormonal, and pathological mechanisms. Osteoinductive factors as growth factors [e.g., bone morphogenetic proteins (BMP), insulin-like growth factors, and transforming growth factors (TGF)] stimulate bone regeneration and repair bone faster. Just introduction of these proteins do not produce expected results, as they diffuse faster. Hence, controlled delivery of growth factors and an environment that is osteoinductive/osteoconductive could be expected to provide better bone growth.

Others

Other classes of drugs as anti-inflammatory (38,39), anticancer (37), and hormones (40) have been tried with success for the local delivery to bone with composites. Nonsteroidal anti-inflammatory drugs have widespread application in orthopedics to inhibit heterotopic ossification and control postoperative pain, inflammatory arthritis, and osteoarthritis. Applications of nonsteroidal anti-inflammatory drugs, such as ibuprofen and aspirin, for local delivery with composites have been reported with success (38,39). Deficiencies of systemic hormone, e.g., estrogen, quite common in women after menopause, significantly alter the strength of the bone. Low estrogen levels are the major cause for osteoporosis that results in brittle bones, highly susceptible to fracture. As the local area would already be brittle, placing a composite that could also support the area in addition to delivering estrogen for prolonged periods would be the rational approach. Osteosarcoma is the most common type of bone tumor, and bone is frequently the first site and the only site of breast cancer at recurrence (37). Cancer demands local delivery and for prolonged periods. Most osteosarcomas are treated with surgery followed by chemotherapy. With composites filling the bone void and supporting the local area with anticancer drugs like paclitaxtel released locally for prolonged periods could move cancer therapy a step forward.

FABRICATION

Fabrications are aimed to satisfy two requirements.

1. Fabrication of suitable scaffold for bone regeneration/support and
2. Incorporation and subsequent release of drug in a predestined schedule

With numerous choices of materials to play with, composite materials widen the choices for design of drug delivery system. Composites could be fabricated by:

1. Organic and inorganic components mixed directly and processed to form composite (21,26,28,32)

2. Preformed structures such as microspheres/beads/blocks made with either of the composite components mixed with the other to form a matrix composite (24,48)
3. Organic or inorganic preformed structures coated with composite materials (15,29).

The structures are prepared by various techniques and are designed to be nonporous (21,24,26) or porous (20,22,23). These varied choices in forming a composite bring with consideration for several parameters (e.g., components composition, bioactivity, osteoconductivity, osteoinductivity, resorbability, drug releasability, mechanical property, stability *etc.*) during design and fabrication of drug delivery systems.

Drugs can be loaded by:

1. Direct mixing with the basic components and then processed to form composite structures (33,36,39,49–51)
2. Entrapping with one of the basic components (as formation of microspheres) and then adding to other components for processing (24,48,52–55)
3. Mixing with a solution of polymer or composite and coating it to preformed structures made of one of the components (15,20,29)
4. Impregnating or adsorbing on preformed structures (27,30,31,56)

Of the methods stated, drugs that are loaded by impregnation or adsorption to preformed structures (27,56) in general exhibit a greater burst release than other methods. Drugs that are entrapped in one of the basic components of the composite (e.g., in polymeric microspheres) (52,53) exhibit lesser tendency for burst release, due to their accessibility limitations to release medium. Schnieders *et al.* reported that burst releases of drug exhibited by polymeric microspheres were not observed with composites (24).

Compression

Compression is the simplest technique in fabrication. In the fabrication of organic–inorganic composite systems, raw, preformed (microspheres) (24), or pretreated (coagulated/precipitated) materials (28) are mixed and compressed with (24) or without (28) the application of heat. Compressed structures made by a single phase of material can be coated with the other phase to form a composite (20).

Castro *et al.* prepared composite by simple mixing and compression (57). PLA, phosphates (Hap/TCP), and drug were mixed and pressed in carver hydraulic press at 520 or 312 MPa for 5 min. Although such fabrications would release drug for prolonged periods, the effect of compression force on drug release would be significant. The authors reported that the higher the compression force, the slower the release of the drug.

Kim *et al.* prepared phosphate glass–PCL composites via solvent extraction and thermal pressing techniques (28). PCL and drug solution in dimethyl carbonate (DMC) was added with phosphate glass dispersion in DMC containing drug and mixed well. The mixture was added to absolute ethanol and stirred vigorously to extract the solvent. The coagulated samples were dried and finally pressed under a load of 100 kg at 80°C. Compared to PCL, glass–PCL composites exhibited enhanced degradation and controlled drug release due to high capacity water uptake and different dissolution rates of glasses depending on the composition.

Schnieders *et al.* prepared calcium phosphate–polylactide-*co*-glycolide (PLGA) composite bone cement by compressing preformed organic phase with inorganics (24). Drug-loaded PLGA microspheres were prepared by spray-drying process. The microspheres were then incorporated into apatitic cement matrix made from tetracalcium phosphate and dicalcium phosphate anhydrate and biaxially compressed at 2.7 MPa for 5 s, followed by a load of 700 kPa for 2 h at 37°C to result composite cylinders. However, the addition of drug-loaded microspheres into apatitic cement and pressing influence a range of properties as drug release, compressive strength and setting time.

Miyai *et al.* 2008 prepared composite discs similarly with β-TCP and polycaprolactone (PCL) by compression molding and coating (20). Porous β-TCP discs were prepared by a solvent-free process in which no toxic solvent was used. β-TCP slurry was poured on a network of stainless steel needles and subjected to a pressure of 28 MPa. After compression molding and drying, the compacts were sintered (1,100°C for 1 h) and finally dipped in molten PCL-containing drug. Coating inorganic scaffolds with organic substrates containing drug influences the mechanical property of the scaffold and the drug release profile.

Melt Compounding

Melt compounding is another popular technique in fabrication. Organic and inorganic components of the composite are mixed with the drug, melted, and formed into shapes on cooling. Koort *et al.* (49) and Makinen *et al.* (26) prepared composites containing ciprofloxacin in a BG-biodegradable matrix. While PDLLA was applied by the former, the latter had PLGA. Fixing a melting temperature could be one crucial step. Although the temperature shall be chosen below the degradation temperature of the drug, a low temperature could cause uneven distribution of the drug in the composite due to incomplete melting of polymer and high viscosity. On the other hand higher temperature could degrade the drug as there could be some phase transformation or complex formation on getting mixed with the components of the composite. A differential scanning calorimetry of the compound to be melted shall help to alleviate the problems.

Cross-Linking/Clotting/Polymerization

Cross-linking is the process of chemically joining two or more molecules by covalent or ionic bonds. Yaylaoglu *et al.* cross-linked composite membranes prepared by solvent casting (58). A mixture of gelatin, drug (gentamicin), calcium phosphate, and water were warmed for an hour, vortexed, and poured in a Petri plate to form a membrane on cooling. Rectangular pieces were cut and cross-linked with glutaraldehyde solution. During cross-linking substantial decrease in drug content could occur due to the leaching of drug. However, leaching was reduced by creating higher diffusional restrictions as increasing the concentration of polymer.

Kelpke *et al.* prepared composites by clotting (59). Human fibrinogen and varying amounts of bovine-derived Hap were mixed with recombinant fibroblast growth factor and clotting achieved by the addition of human thrombin. Fibrin and fibrinogen degradation products function as a scaffold for migrating fibroblasts, stimulate soft tissue cell growth, accelerate vascular sprouting, induce growth factor release from responding cells, and hence suitable as a biodegradable delivery system for various growth factors. Fibroblast growth factors undergo thermal degradation at 37°C, resulting to a short biological half-life *in vitro*. Kelpke *et al.* reported that fibroblast growth factor was protected against thermal degradation and inactivity by clotting.

Composites prepared by polymerization are quite often those containing PMMA. Composites containing alumina, drug, PLA, and PMMA were prepared by free radical polymerization with benzoyl peroxide as initiator (60). Due to their mutual interaction through polar coupling and hydrogen bonding, there exists good adhesion between alumina–polymer associates. Composites containing a mixture of polymer, glass, and drug by free radical polymerization were prepared by Ragel *et al.* (33). In short, methyl methacrylate/benzoyl peroxide solution was added to polymethylmethacrylate (PMMA). PLA followed by glass premixed with drug was added and formed into parallelepipeds by forcing the mixture into Teflon molds and dried at 60°C for 24 h. Padilla *et al.* (50) prepared composites in a similar procedure with Hap, gentamicin, PMMA, and poly(ethyl methacrylate). Composite microspheres by dispersion polymerization technique containing coralline Hap and gelatin were prepared by Sivakumar *et al.* (31). A mixture containing coralline Hap and gelatin solution was added drop by drop into PMMA dispersion solution with constant stirring, resulting to the formation of microspheres. Glutaraldehyde saturated toluene was added as cross-linking agent. After cross-linking, PMMA was washed out by several installments of solvents with toluene, acetone, and finally water. Loading of drug was performed by immersing the composite microspheres in phosphate-buffered saline containing drug.

Drug can be loaded either with the raw materials before polymerization or after formation of composites. However, stability of the drug should be considered as rise in temperature during polymerization of PMMA reportedly range from 80°C to 124°C (61,62). In addition to the processing conditions, components of the composite themselves may influence drug properties. For example, ibuprofen shifts from amorphous to crystalline state due to the presence of α-Al_2O_3 together with PLA, resulting in faster release (60).

Emulsion–Solvent Evaporation Method

Preparation of polymeric microspheres by emulsion–solvent evaporation method is a popular technique. Oil phase (usually an organic solution of polymer) is dispersed in an aqueous phase containing an emulsifier and the dispersion stirred or ultrasonicated for prolonged periods for evaporation of organic solvent and formation of microspheres. In such a technique, the addition of inorganic phase with the organic phase will result in the formation of composite microspheres. Li *et al.* (27) prepared organic–inorganic composite microspheres by this method. Wollastonite powders were mixed with organic solution of PHBV and the mixture stirred for 2 h to gain a homogeneous mixture. The PHBV/wollastonite mixture was then added dropwise into 1% polyvinyl alcohol solution. The mixture was vigorously stirred for 24 h to allow complete solvent evaporation. The resulting microspheres were washed twice with deionized water and then collected by filtering. After that, these microspheres were lyophilized to dry. Drug loading was performed by adsorption technique by immersing the microspheres in drug solution for 24 h. A modified version was employed by Wang *et al.* (54). Drug-loaded Hap nanoparticles were dispersed in an organic solution of PHBV and polyethylene glycol (PEG) by ball milling. The resulting mixture was added into water with methyl cellulose as emulsifier and the resulting emulsion stirred for few hours to evaporate the organic solvent. The collected microspheres were finally freeze-dried. The addition of PEG could improve the permeability of the polymer and surface property of microspheres.

In addition to the simple emulsion–solvent evaporation process, composites can also be prepared by multiple (w/o/w) emulsion–solvent evaporation (55). Hap powder and drug (BSA) were dispersed in phosphate-buffered saline (PBS) buffer to form the first water phase. PLGA in an organic solvent served as the oil phase. The first water phase and oil phase were mixed at 1,000 rpm to form w/o simple emulsion. The first emulsion was added to an aqueous solution of PVA and stirred at 500 rpm for a few hours to form a multiple emulsion (w/o/w) and drive off organic solvent and solidify the microspheres. The microspheres were collected and finally freeze-dried. As compared to the earlier examples stated here, drug could be added initially in this case. However, the final drug amount present would heavily depend on the encapsulation efficiency of the process. In addition, the release of drug from these microspheres would depend on the rate of polymer degradation in addition to drug diffusion through pores of microspheres.

Freeze Drying

Freeze drying or lyophilisation is a drying technique that freezes the solvent present in the material and sublimes it, resulting to a material subjected to less damage as compared to other drying processes, which usually employ high temperatures. Hence, freeze drying could be a better option when the process involves thermolabile materials as enzymes, proteins, *etc.* In addition, freeze drying does not usually cause shrinking or toughening of the material and results with a microporous structure. Drug can be incorporated either during homogenization step (63) or by adsorption (14,30) technique before (14) or after (23,30) freeze drying.

Martins *et al.* (63) homogenized Hap and anionic collagen. Drug was added to the composite mass and lyophilized. Zhang and Zhang (30) applied a similar procedure with invert calcium phosphate and β-TCP as inorganic phase and chitosan as organic phase. However, drug loading was done by adsorption technique with lyophilized sections of the composite. Lee *et al.* (14) prepared composite microgranules with chitosan and TCP. TCP was added to chitosan solution and added dropwise into a coagulation solution of a

4% NaOH ethanol/water mixture. The resultant microgranules were washed and freeze-dried. TGF was incorporated by adding the composite microgranules into a solution of TGF-β1 and stored overnight at 4°C before freeze-drying. Zhang *et al*. reported the preparation of composite scaffold by freeze-drying (64). Invert calcium phosphate glass and Hap powders were mixed into chitosan solution to make a composite mixture. The resultant mixture was frozen and freeze-dried for 4 days to obtain composite scaffolds. By a similar procedure, Gravel *et al*. (65) prepared chitosan–coralline powder composite. Although systems obtained by freeze-drying technique are porous, additional porosity as needed can be obtained by salt-leaching technique (23). Zhang *et al*. (23) prepared PDLLA/biphasic calcium phosphate scaffolds by salt-leaching technique. PDLLA was added to biphasic calcium phosphate dispersed in 1,4 dioxane–water solvent and mixed to result a gel. Ammonium bicarbonate salt particles were added to the gel, cast and freeze-dried. The resultant products were immersed in distilled water to leach out the salt and dipped in PEG–drug mixtures.

Freeze Gelation

Freeze gelation method can be used to prepare highly porous scaffolds without using the time and energy consuming freeze-drying process. The porous structure was generated during the freeze of a polymer solution, following which the polymer was gelled under freezing condition so that the porous structure would not be destructed during the subsequent drying stage (66). Hsieh *et al*. (44) reported preparation of chitosan/γ-PGA solution by freeze gelation process. In this method, the frozen scaffold solution (chitosan/γ-PGA) was immersed in a gelation solution (3 M NaOH/ethanol solution) at a temperature lower than its freezing point (−20°C). The scaffold had already gelled before the drying stage; thus, the porous structure could also be retained without lyophilization by this method. Hsieh *et al*. (44) also reported that the freeze-gelled composites exhibited a better profile for rhBMP-2 as compared to freeze-dried scaffolds. Although this process has been compared to freeze drying, it is a form of sol–gel processing that enables ceramic fabrication without the need for high temperature sintering.

Sol–gel

There is considerable interest in organic–inorganic hybrid composite materials prepared via sol–gel process. First, a homogeneous sol is prepared, which transforms to a gel, followed by controlled drying to obtain a monolith or powder. The sol–gel chemistry is based on the hydrolysis and polycondensation of metal alkoxides M(O*R*)*x*, where M = Si, Zr, Ti, Al, *etc*. and R = any alkyl group. The first reaction is a hydrolysis, which induces the substitution of OR groups linked to M by M–OH groups. As previously, these chemical species may react together to form M–O–M bonds, which lead to the formation of metal network. This phase establishes a three-dimensional network with solvent applied in these reactions resting within the pores of the network. Catauro *et al*. (35) prepared organic–inorganic hybrid composite materials via sol–gel process. With PCL and TiO_2 glass serving as organic and inorganic phases, the drug was added during the sol–gel process. A similar procedure was adapted to prepare composite films with PMMA and silica by Lin *et al*. (39) and PCL and zirconia by Catauro *et al*. (36). However, the sol–gel reactions are affected by many parameters, such as structure and concentration of the reactants, solvents, and catalysts, reaction temperature, rate of removal of solvents, and the drug release rate by content of inorganics and coupling agent (36,39). The process is slow and involves usage of toxic chemicals.

Fused Deposition

Fused deposition represents a rapid prototyping process that can make custom specific structures. Fused deposition permits one to design and fabricate scaffolds with a completely interconnected pore network, highly regular and reproducible scaffold morphology, a microstructure that can be varied across the scaffold matrix, and a solvent-free process (67). Fused ingredients are delivered through a moving nozzle on a moving platform. A suitable computer program (CAD/CAM design or.stl file) that decides the composition of ingredients, degree of fusing, and the design of the final structure of the required implant dictates the movement of nozzle and platform. As the process involves fusing, this technique might not be possible for loading the drugs in matrix with basic components that make the composite. However, being a solvent-free process, the ill effects of residual solvents that could affect the stability of growth factors get shoved away. Rai *et al*. (22) prepared PCL-TCP scaffolds by fused deposition modeling with a porosity of 65%. Scaffolds were soaked in PBS for 3 h at 37°C before rhBMP seeding. Drug loading was performed by adding the drug to fibrin and then loading it to the scaffold. On comparison of polymeric and organic–inorganic composite scaffolds, the authors reported that composites exhibited comparatively lesser drug loading and higher drug release on day 1. These results relate to the property of TCP. The presence of TCP improves hydrophillicity, causing more dissolution medium penetration and faster diffusion of drug.

Sponge Reticulate Method

Methods to prepare porous ceramics can be divided into two groups based on pore structure: One is a foam structure in which closed pores are dispersed in the matrix; the other is a reticulate structure, in which open pores are interconnected through channels (68). A reticulate structure is obtained by filling a sponge with slurry of the material and subjecting them to higher temperatures to burnout the sponge, resulting in a porous dense ceramic block. Once an inorganic porous block is obtained, organic polymers can be filled into pores to form composites (29,69).

Tampieri *et al*. reported preparing composites with a porous Hap skeleton and gelatin filling mass (69). Porous inorganic skeleton was prepared by soaking cellulose sponges with slurry of Hap and sintering at 1,250°C. The

sintered bodies were immersed in gelatin solution, and a slow suction under vacuum was applied to coat and fill the porous structures. They were then air-dried and treated with glutaraldehyde to cross-link gelatin. Kim *et al.* (29) followed a similar technique with some modifications. With polyurethane sponge instead of cellulose sponge to form porous Hap structures, the filling and coating material applied was a composite. Porous structures were dipped in drug containing composite solution made of PCL and Hap in dichloromethane. Coating a porous drug-loaded inorganic structure with a polymeric solution could be very pleasing to control drug release. However, the same polymeric coating may result as an obstacle for new bone formation and bone in-growth. Pore morphology (pore connectivity and percent porosity) and pore size play critical role in rendering Hap ceramics osteoconductivity and osteoinductivity (70,71). Coating such structures with polymer locks the micropores and reduces the size of macropores for a period of time, as governed by the polymers degradation, affecting the structures' osteoconductivity. A coating by glycerol-L-lactide on porous Hap structure (Fig. 2) caused significant delay in bone in-growth as compared to uncoated structures (72).

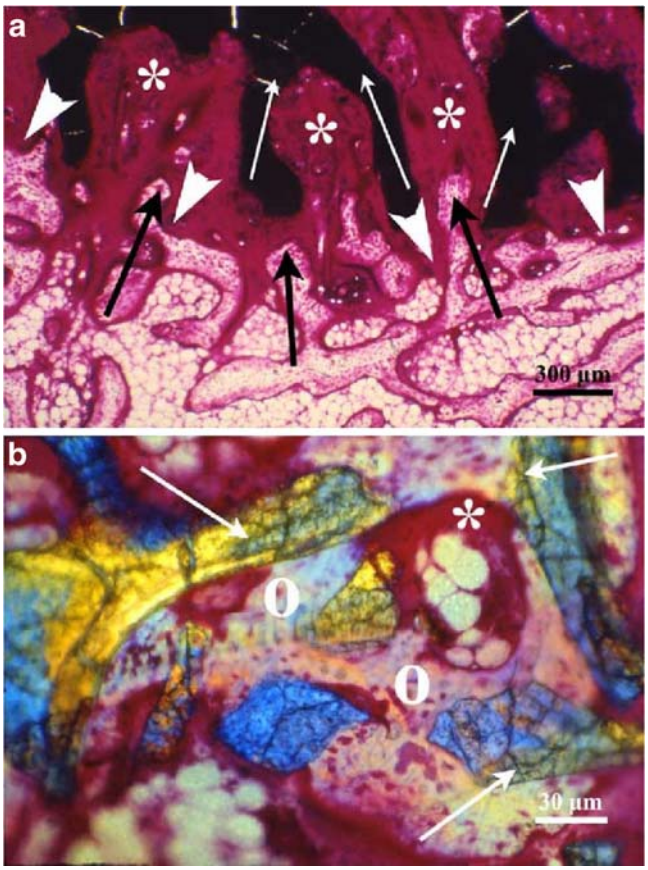


Fig. 2. The basic fuchsin histology of polymer-coated ceramic implants subjected to *in vivo* studies. **a** Polymer (asterisk) filling the Hap pores (*white arrows*) of the composite (*black arrows*) and acting as barrier for osteoblasts, resulting in no bone in-growth after 42 days. **b** Fluorescence-stained pores (*white arrows*) were opened after polymer degradation, and new bone formation and bone in-growth (*circle*) has happened after 84 days (72) (Reproduced with permission)

PROPERTIES

Mechanical Properties

For a successful bone grafting, bone substitutes are expected to mimic the mechanical properties of the surrounding bone. Higher values shall render the surrounding bone weak, and lower values shall provide ineffective support, leading to chances of further damage. The average compressive strength and torsion strength of bone ranges from 167 to 145 MPa and 57 to 49 MPa, respectively, depending on age (73). Causa *et al.* (23) reported that the elastic modulus of PCL increased from 8.25 to 27.9 MPa by the addition of Hap, and the compressive strength and compressive modulus of organic (PDLLA) scaffolds doubled by the addition of inorganics biphasic calcium phosphate resulting as composites. It is obvious that, in a composite, the mechanical property of one component shall influence that of the other, and the addition of drugs is no exception. Negative effects of drugs on mechanical properties of cement have been reported (74). Addition of drug to calcium phosphate bone cements shall influence the latter's setting time and compressive strength. However, by moving to an organic–inorganic composite, neither the setting time nor compressive strength is altered significantly. Schnieders *et al.* (24) reported that addition of gentamicin crobefate to calcium phosphate cement abolished cement setting, and gentamicin sulfate addition increased the setting time up to 26 min against the acceptable limit of 12–15 min. In contrast, there was no or acceptable change of setting time when the drug-loaded PLGA microspheres were added to the cement resulting in a PLGA/calcium phosphate cement composite possibly due to PLGA acting as a barrier between the drug and cement. In addition, the compressive strength of calcium phosphate cement increased significantly up to 70 MPa from 52 MPa by the addition of PLGA microspheres containing drug. Kim *et al.* (29) reported that composites fabricated by coating can improve its mechanical properties significantly. Pure Hap porous scaffolds were compared with those coated with a mixture of Hap and PCL-containing drug. They reported that, as compared to pure Hap, coated samples exhibited higher compressive strength and elastic modulus, with little influence from coating composition ratios. These studies suggest that formation of a composite shall influence the mechanical features as compressive strength, compressive modulus, elastic modulus, and setting time. On the other hand, they also provide opportunities for tailoring the properties of the resultant composite by controlling the processing ingredients as well as the fabrication or processing parameters. Custom-specific implant fabrication is also possible using these inorganic–organic composites.

Drug Release

The release of drug from a carrier system depends on several factors. Primarily, it would be the area of exposure and dissolution pattern if the drug was present in a matrix as with polymers and the type of complex or bond it forms if present adsorbed on the surface as with glass and ceramics. In case of composites, the presence of one component shall also

influence the property of another on drug release. As such a case, Tuzuner *et al.* (21) reported that teicoplanin release from CS-Versa Bond™ composite was better when compared to its release from Versa Bond™ alone. In another case, a β-TCP, calcium phosphate invert glass, chitosan composite exhibited a better gentamicin release profile than a chitosan matrix (30). The addition of β-TCP and calcium phosphate invert glass to a chitosan matrix greatly reduced the burst release of gentamicin followed by a higher and steadier release profile. While the dissolution of β-TCP and calcium phosphate invert glass increased the phosphate concentration in the dissolution medium that improved the cross-linking of chitosan, calcium and sodium ions restricted the enhanced dissolution of chitosan acetate by buffering the local dissolution medium, thereby restricting the release of gentamicin. In addition, the apatite-forming ability of a composite shall also influence drug release. It has been reported that apatite formation sustained the release of drug by about threefold in simulated body fluid (SBF) (27). Another significant advantage that a composite may offer for drug delivery is linear release kinetics (24,31). Schnieders *et al.* reported that the release of gentamicin crobefate from a PLGA microsphere/calcium phosphate cement composite followed a nearly zero-order kinetic, which was characterized by a slower but linear release over 100 days without initial drug burst against triphasic drug release kinetic from PLGA microsphere or plain drug-loaded bone cement (24). A sustained release from PLGA microspheres followed possibly by adsorption of the released drug on calcium phosphate cement matrix, and slower drug diffusion out of it resulted to a linear drug release profile. In addition to a stable *in vitro* release, it is also mandatory to know whether the released drug for prolonged period retains its antimicrobial property. Makinen *et al.* (26) reported that the antibacterial activity of released ciprofloxacin from composite, after manufacturing and sterilization processes, was similar to the non-processed ciprofloxacin throughout the process of release. Finally, in a conventional sustained release therapy, there shall exist a slow residual release with suboptimal concentrations. Such a release shall not be a point of discussion with most classes of drugs, however, not with antibiotics, as suboptimal release of antibiotics might cause chances for development of drug resistance in microorganisms. However, Makinen *et al.* (26) reported that, with composites, the release profile appeared safer, avoiding worries related with drug resistance.

Many studies have reported the kinetics and mechanism of drug release from various composite systems. It appears that the drug release mechanism from a composite is not simple diffusion alone, and it gets influenced by various other factors, such as ion exchange between composite and SBF, formation of bioactive component (apatite) on the surface of composite, and or dissociation of drug from the complex formed with the component of composite (18,27,32,33). Researchers (27,32,33) observed that the fraction of the drug released from a heterogeneous parallelepiped-shaped, insoluble (partially in these cases), porous composite *versus* square root of time fitted to a third order polynomial and the value of release rate constant (K_b) could be obtained by applying the experimental values in the following equation $f_t = \frac{(a_0b_0+a_0c_0+b_0c_0)}{V_0} K_b t^{1/2} - \frac{(a_0+b_0+c_0)}{V_0} K_b^2 t + \frac{1}{V_0} K_b^3 t^{3/2}$ where f_t is the fraction of drug released at time (t), K_b the boundary retreat rate constant, a_0, b_0, and c_0 the parallelepiped dimensions, and V_0 the parallelepiped volume. With reduced burst release, higher and sustained release with linear drug release kinetics organic–inorganic composites exhibit desirable drug release profiles suitable for local drug delivery in bone. The drug release profile can be theoretically modeled and can be of great help in the design of drug-releasing scaffolds applied for the treatment of certain chronic diseases like osteomyelitis, where controlled local delivery of drug is required for a relatively longer period.

Biocompatibility

Although biologically acceptable ingredients are present in the final form of composite, a problem of bio non-compatibility could arise due to the presence of trace amounts of solvents, monomers released from polymers, or due to processing factors. The biocompatibilities of composite scaffolds are studied by *in vitro* cell culture method by employing osteoblast-like human osteosarcoma cell lines as MG63. The cell growth on the surface and migration into pores of the composite, if any, and their morphology and attachment all indicate biocompatibility and suitability of the material for *in vivo* use (30). Cell proliferation is popularly observed by 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay (23,28) or fluorescence micrograph (23). Zhang *et al.* observed the influence of invert calcium phosphate glass and β-TCP on the biocompatibility of chitosan scaffolds (30). They observed that MG63 cells attached and grew with similar morphology on composite scaffolds and chitosan scaffolds. This indicates that addition of new (biocompatible) ingredients to a biocompatible substance need not necessarily influence the biocompatibility. However, this study does not mention the composition of the composite scaffold subjected to the cell culture study, and hence, its result better dealt with caution.

Zhang *et al.* (23) employed ROS 17/2.8 rat osteoblastic cell lines for biocompatibility studies for PDLLA/BCP composite scaffolds and those coated with PEG and PEG–vancomycin. Cell proliferation was observed by fluorescence micrographs and MTT assay. Figure 3 displays the fluorescence micrograph of the studied samples on days 1 and 6 (23). The results showed that cells attached and continue to proliferate in all samples, indicating that PEG and vancomycin coating had no negative effect, and all three had good biocompatibility. Alternatively, cell responses to composites can be assessed with the liquid extracts of composites (28). Kim *et al.* (28) studied the biocompatibility of phosphate glass–PCL-based composites. Two varieties of phosphate glass were used, which differed on their CaO content, thereby influencing the solubility of glasses. MG63 cells proliferated well on the extracts. When compared to the composite containing highly dissolvable glass (with lower CaO content), those with less soluble glass (with higher CaO content) showed a higher proliferation level. As the pH of extracts was maintained uniformly, the difference in cell proliferation could be attributed to influence of ionic range appropriate for cellular activity. The biocompatibility studies on composites are very limited. However, the tested composites have been reported to be biocompatible (28,30). There is ample scope of

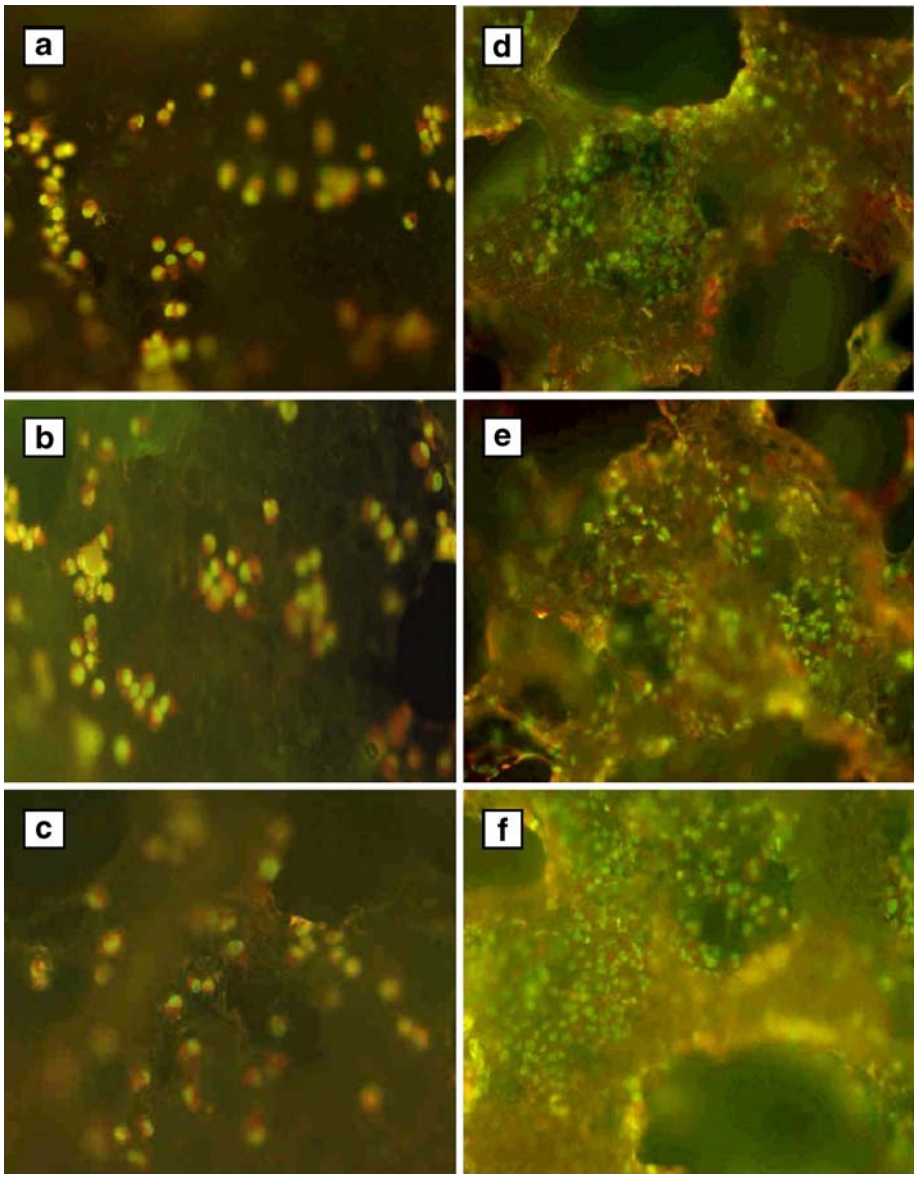


Fig. 3. Fluorescent micrograph of the osteoblast cultured on PDLLA/BCP scaffolds without coating (**a**, **d**), with PEG coating (**b**, **e**), with PEG–vancomycin coating (**c**, **f**). **a–c** Day 1; **d–f** day 6 (23) (Reproduced with permission)

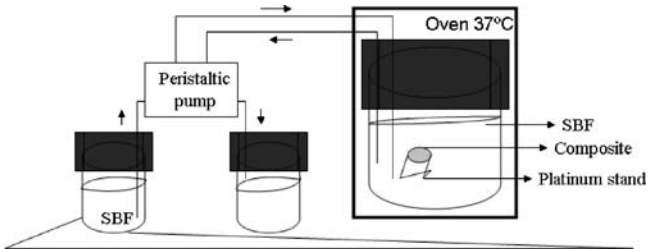
future research in this area. A study on biocompatibility of these composites should be considered mandatory than optional in research.

Bioactivity

The bioactivity of a composite and its individual components can be assessed *in vitro* (32,33,35,36,49) by their ability to form apatite layer on surface overcoming the possible interference of polymer and drug if any. *In vitro* bioactivity studies are conducted by immersing the samples in SBF, which have the composition and ionic concentration similar to human plasma, at 37°C (Fig. 4). As a result of surface interaction with SBF, a layer of Hap starts to form on the surface. However, the crystallinity of the apatite phase formed on the composite surface may be lower than that formed on the surface of individual bioactive component during the same period. The formation of apatite can be analyzed by X-ray diffraction, scanning electron microscopy, X-ray energy dispersive spectroscopy, and/or with Fourier transform infrared (32,33). With concern on animal usage in experimentation, the *in vitro* method for bioactivity study gains more appreciation. Standards for *in vitro* measurement of apatite-forming abilities on implant materials (ISO/DIS 23317) are in the development stage by the International Organization for Standardization.

Biodegradation/Bioresorption

Biodegradation is the breakdown of macromolecules by the action of a living system or via enzymes and thus cells, and bioresorption is the elimination of byproducts of biodegradation from an animal organism via natural pathways. Biodegradation of drug-releasing composites is studied



Ionic composition (mM) of SBF in comparison with human plasma

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
Plasma	142.0	5.0	1.5	2.5	103	27.0	1.0	0.5
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5

Fig. 4. Schematic depiction of *in vitro* bioactivity test based on dynamic protocol and ionic composition of SBF in comparison with human plasma

in vitro by observing the decrease in weight of the sample and molecular weight of the degrading polymer after immersion in a medium at 37°C for various periods of time with liquids as sterile pH 7.4 PBS solution (28,29,53,56,75) or SBF (75) acting as medium for degradation. Although both the liquids are not very appropriate in simulating the *in vivo* environment, SBF is a better choice than PBS. SBF simulates pH and ionic concentrations more closely as plasma (Fig. 4) and also imparts bioactivity to the sample in study. Hence, when a composite is subjected to biodegradation studies, the one in SBF will also exhibit bioactivity and influence the degradation simulating better *in vivo* condition.

In general, the weight loss of the composite scaffolds is monitored over a period of time. In addition, studies on change in molecular weight of the polymer determined by gel permeation chromatography (28) and the concentration of Ca and P released from the composites determined by inductively coupled plasma atomic emission spectroscopy (28) do exist. If the structure is porous, changes in pore morphology can be observed by SEM. As a result of hydrolytic degradation, the decrease in weight of the composite could be monophasic (almost linear weight loss with time) (28), biphasic (fast initial loss further decreased loss) (28), or triphasic (fast initial and terminal with decreased intermediate) (53), depending on the composition of the composite materials. In the case of composites prepared by coating, thickness of the coat influences the degradation as well (29).

Kim *et al*. observed that the degradation of PCL–phosphate glass composites was heavily influenced by variation of CaO concentrations in a P2O5–CaO–Na2O glass system, though the organic to inorganic phase ratio remained constant (28). It was observed that the concentration of CaO in the glass and the degradation were inversely proportional. The lower the CaO, the faster is the weight loss, and the higher the Cao, the slower is the weight loss of the composite. The composites with high CaO glass exhibited almost linear weight loss with time, while other compositions showed a fast initial loss and further decreased rate. Although addition of glass increased the weight loss of the scaffold, this change in trend based on CaO concentration could be attributed to the solubility of the glass, as higher CaO concentrations are observed to lower the solubility of P2O5–CaO–Na2O glasses (76,77). However, this study was conducted for only 7 days, and hence, the degradation of PCL and its influence in this degradation cannot be assumed to be substantial. The influence of slower degrading polymers can be observed only when studied for prolonged periods of time.

The composites of Niu *et al*. made with nano-Hap-collagen-PLLA exhibited a three-phase weight loss with the introduction of cross-linked chitosan microspheres and the weight loss proportional to the chitosan microsphere proportion in the composite (53). Figure 5 depicts the three-phase weight loss curves of the studied composite with varying proportions of chitosan microspheres (53). The three-phase trend is more pronounced with higher proportions (30% and 50%) of chitosan microspheres. The rate of weight loss was higher during the first phase (first 3 weeks) and third phase (14–18 weeks) due to preferential dissolution of chitosan microspheres and degradation of PLLA, respectively. In the second phase, the degradation rather slowed down. In common, the degradation of most synthetic semicrystalline polymers as PLLA that undergo hydrolytic degradation exhibits two phases. In the first phase, water diffuses into the polymer that converts the long polymer chains into shorter ones. As this occurs in the amorphous region, there is reduction in molecular weight but no loss in physical properties, while the crystalline regions hold the structure together. In the second phase, crystalline regions fragment and the related physical properties diminish with the fragments metabolized *in vivo* by enzymes, resulting in a rapid loss of polymer mass (78). Hence, it could be concluded that the period for *in vitro* studies on biodegradation or bioresorption be decided based on the components of the composite studied and not arbitrarily.

IN VIVO ASSESSMENT

In vivo assessment of a composite is not just restricted to drug release but extended to its bioactivity. *In vivo* studies are carried out in rabbits following radical debridment of localized osteomyelitis and drug concentrations estimated by extracting the drug from bone specimens (26). Generally, the

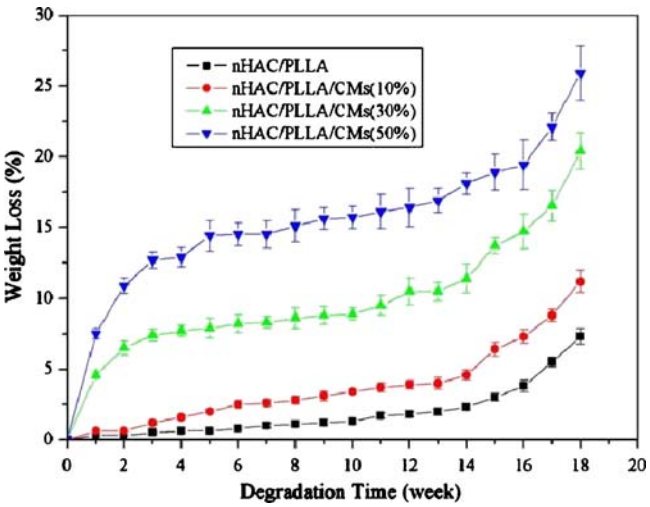


Fig. 5. Weight loss of nano-Hap-PLLA-Chitosan microspheres scaffolds with different chitosan microspheres contents during hydrolytic degradation (53) (Reproduced with permission)

drug concentration was high in and around the areas in which the composites were in contact, and the concentration decreased as the distance increased. Therefore, under clinical conditions of use, there could be an obvious need for systemic administration of drugs to protect the surrounding bones and tissues, at least for a limited period initially. Otherwise, composites appear suitable enough in releasing the drugs and in their active form throughout the study period (26). However, it is not a case unique to composites, and similar conditions are exhibited by drug delivery systems either prepared by the polymer or inorganic. *In vivo* biodegradation and concomitant new bone formation can be studied by means of sequential peripheral quantitative computed tomography imaging of the density and area of trabecular and cortical bone. The decrease in cortical bone area as a function of time with a concurrent increase in its density is an indication of bone remodeling response. Miyai *et al.* (20) reported the new bone formation and osteoconduction of composites. After 50 weeks of study in rabbit, the composites were surrounded by mature bone. In-growth of thin bone tissues was also observed along the inner wall of the pores of the ceramic, which indicated degradation of the polymer and/or ceramic at the ceramic/polymer interface and subsequent replacement by the thin bone tissues. In some pores, bone tissue with vascular channels was also formed. These studies assure the ability of the composites in delivering drug locally and biointeraction with local tissue, leading to new bone formation. However, due to ethical issues, experimental design, and biological and study conditions, *in vivo* results may be highly influenced by statistics, and in the case of organic–inorganic composites, the reports are relatively few.

CONCLUSION

Application of individual components such as polymer or ceramic for bone filling and drug release is in practice for a couple of decades. In addition, the idea of application of a composite in the same area is picking up faster. With reports assuring the ability of composites to deliver drug and favor new bone formation with acceptable mechanical properties and biocompatibility, composites have a major role in tissue engineering and drug delivery for bone. However, there are still many questions to be answered. Not all composites behave in the same manner and not two compositions of the same composite in releasing drug. In addition, the manufacturing technique also plays its role. Properties like drug delivery and biodegradation are the first that shall get affected by this, and interestingly, they are the key determinants that take a particular composite in or outside the acceptable boundary. If all the initial hurdles were crossed, the final would be industrialization and commercialization. From a tissue engineering perspective, selection of a method that provides the best mechanical strength to the final unit would be more important. However, from a pharmaceutical perspective, the conditions like temperature and pressure that may favor drug decomposition shall restrict the selection. Newer techniques as rapid prototyping would help to make structures that have the best uniformity and reproducibility as far as even distribution of drug and porosity are considered. Nevertheless, mechanical ability of those structures to compete with structures made by compression as Iso Static Press is to be considered. At present, the market segment for these kinds of systems is relatively small worldwide. Hence, at the present juncture, it would be better to form composite structures that are more suitable in space filling and new bone formation, with provisions for drug incorporation at later stages. The drug choice can be left to the surgeon who could incorporate the drug into composite prior to surgery as the case desires. Such a path would provide a broader market potential than a single drug fixed dose composition in a composite.

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