Pharm Res (2011) 28:1224–1228
DOI 10.1007/s11095-010-0359-4

COMMENTARY

Sintered Microsphere Scaffolds for Controlled Release and Tissue Engineering

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Received: 20 October 2010 / Accepted: 20 December 2010 / Published online: 7 January 2011

KEY WORDS controlled release · microsphere · sinter · tissue engineering

Scaffolds are an essential component in tissue engineering, as they provide a three-dimensional niche for cell residence and tissue regeneration (1). In recent decades, further incorporation of controlled release functionalities into newly designed scaffolds, in pursuit of better regulation and promotion of tissue development, has drawn more and more attention. Sintered microsphere scaffolds (SMSs), first developed in 2001 (2), manage to combine the excellence of controlled release functions, which are inherited from common degradable microspheres, and the talent of cell delivery, which belongs to generic porous scaffolds, so that unique superiorities are assembled together. Besides the capacity of controlled release, SMSs also exhibit excellent initial mechanical properties. The compressive strength and compressive modulus of polymer-based SMSs are comparable to those of cancellous bone (3).

Currently, protocols for fabrication of SMSs via heating are based on Borden's recipe (4) (Fig. 1). Briefly, pre-fabricated polymer microspheres produced by solvent evaporation technique are poured into a mold and heated to a specific temperature above the glass transition temperature (Tg) of the polymeric matrix for several hours. This results in melting of the surface layer on microspheres and therefore induces bonding of adjacent microspheres into a three-dimensional porous scaffold with excellent mechanical properties (4).

Mechanical properties and porous structure of scaffolds are of great importance for bone repair. SMSs are built up by bonding of microspheres via surface fusion; therefore, sintering temperature and sintering time are generally recognized as crucial determinant factors. Higher sintering temperature and extended sintering time lead to stronger fusion of microspheres, smaller microsphere sizes, lower porosity and also smaller porous diameters, which give rise to a scaffold with better mechanical properties. On the contrary, lower sintering temperature and shorter sintering time induce weaker coalescent among the adjacent microspheres, which results in lower capability of SMSs to resist outside forces. Hence, optimization of sintering conditions would endow the scaffold with excellent characteristics for bony reconstruction, namely, desirable porosity and mechanical strength.

Using a solvent to induce bonding of microspheres is another effective route to fabricate SMSs (5). Different solvents, such as methylene chloride and acetone (5,6), have been used to stick the separated microspheres into SMSs (Fig. 1). In comparison with heat sintering, solvent sintering provides milder means for manufacturing SMSs and thus

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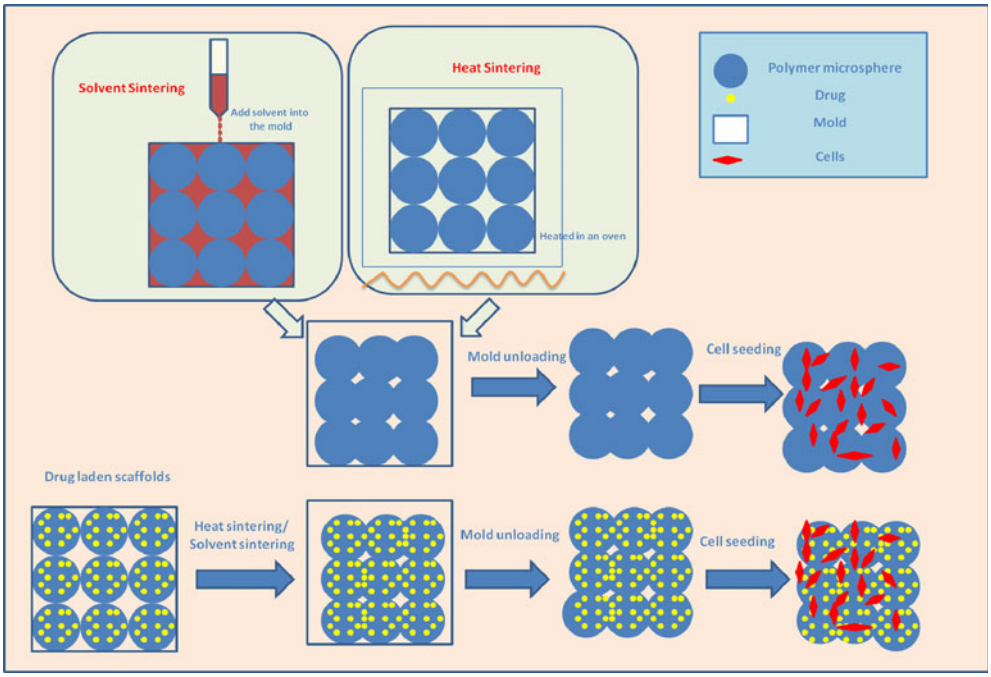

Fig. 1 The scheme of producing cell/drug-laden SMSs by solvent sintering or heat sintering: Drug-laden or plain microspheres were placed into molds, and then a certain solvent was added (solvent sintering) or the whole construct was put into an oven (heat sintering) with predetermined temperature and time period. After sintering, the mold was removed, and the scaffold made by solvent sintering was washed with deionized water. Cell seeding was performed by directly adding cell suspension onto the drug-laden or plain scaffolds.

has broader applications, especially for drug/protein delivery purpose. Factors such as Tg, viscosity, crystallinity and surface tension of the polymer, as well as heating conditions (heating temperature and heating time), must be taken into consideration when fabricating SMSs utilizing heat sintering (5). In contrast, if solvent sintering strategy is adopted, only two factors must be taken into account: species of solvent and sintering time.

Poly(lactide-co-glycolide) (PLGA) has been used most popularly as matrix material for preparation of SMSs via heat sintering because of its excellent biocompatibility and physical/mechanical properties, such as regulated degrading profiles and appropriate Tg (7). Poly(ε-caprolactone) (PCL) (8) has also been employed for scaffold construction in virtue of comparable advantages of PLGAs. With the development of solvent sintering strategy, a broader range of polymers has been utilized as matrix materials for fabrication of SMSs, including polyphosphazenes (9) and chitosan (10) (Table I).

On many occasions, polymers such as PLGA that lack cell-affinitive moieties may be demanded as substrate materials for cell attachment; therefore, modifications of polymeric matrix are required. Natural macromolecules have been employed to improve the bioactivity as well as the bio-processing properties of SMSs. Shi *et al.* have incorporated lecithin into PLGA SMS system (3). Osteoblasts are cultured on the PLGA/lecithin composite scaffolds for 24 days. The results indicate that hybridization with 5% lecithin in PLGA SMSs has remarkably promoted cell viability, bone-related gene expression, and protein secretion. Additionally, introduction of lecithin into PLGA SMSs also largely enhances encapsulation efficiency of highly hydrophilic drugs such as gentamicin sulfate (GS) and bovine serum albumin (BSA). The BSA encapsulation efficiency in PLGA/10 wt % lecithin poses over 50% higher than that in pure PLGA SMSs (11). Jang *et al.* have systematically investigated SMS systems made by hybridizing chitosan in PLGA matrix (12). They find that the composite SMSs favour osteoblastic cells (MC3T3-E1) better in terms of proliferation, maturation, and osteogenic differentiation *in vitro*. *In vivo* results indicate that these composite scaffolds can guide bone reconstruction in rabbit ulnar defective models. Further modification in these PLGA/chitosan SMSs via heparin immobilization has also been performed by Jang *et al.* (13). It demonstrates that the heparin additive works to further improve osteogenic performance of the same osteoblastic cells in bone tissue engineering.

Nano-particles have also been used to promote bioactivity of SMSs for tissue engineering purpose. Hydroxyapatite (HA), with inherently high structural and compositional similarities to bone minerals, has attracted extensive attention for therapeutic applications in bone regeneration (14). Three effective routes have been adopted to introduce HA into PLGA or polyphosphazene SMSs so as to improve the osteoconductivity of SMSs.

1) The simplest strategy is to directly hybridize PLGA matrix with nano HA. Kofron *et al.* (15) have developed tubular PLGA/HA SMSs following this route to mimic the marrow cavity in native bone tissue. Lv *et al.* (16) find that PLGA SMSs with 20 wt % HA sintered at 90°C for 3 hrs possess the highest compressive strength and appropriate porosity for bone tissue repair applications. Nukavarapu *et al.* have chosen 100-nm-sized

Table I Selected Experimental Trials Carried Out for SMS

Methodology	Materials	Cells laden on the scaffolds	Drug/protein laden on the scaffolds	References
Heat sintering	PLGA	Osteoblasts		(2,4)
	PLGA/lecithin	Osteoblasts		(3)
	PCL		BSA	(8)
	Chitosan			(10)
	PLGA/chitosan	Osteoblasts		(12,13)
	PLGA/HA	MSCs		(15)
	PLGA/HA	MSCs		(16)
	PLGA/HA		Ovalbumin	(18)
	PLGA/HA			(19)
	PLGA/BG	MSCs		(20)
	PLGA/BG	Osteoblasts		(21)
	PLGA/MSH	MSCs	GS	(22)
	PLGA	Endothelial cells		(24)
	PLGA	*In vivo*		(25)
	PLGA	MSCs	AL; Dex	(27)
	PLGA	MSCs	Dex; AA; GP	(26)
Solvent sintering	Polyphosphazenes			(5)
	PLGA	Fibroblasts	BSA; IGF-I ; TGF-b1	(6,23)
	PLGA/lecithin	MSCs	BSA; GS	(11)
	Polyphosphazene/HA	Osteoblasts		(9)

PLGA poly(lactide-co-glycolide), *PCL* poly-ε-caprolactone, *BSA* Bovine serum albumin, *GS* gentamicin sulphate, *TGF-β1* transforming growth factor-beta1, *IGF-I* insulin-like growth factor-I, *BG* bioactive glass, *HA* hydroxyapatite, *MSH* mesoporous silica-hydroxyapatite, *AL* alendronate, *Dex* dexamethasone, *AA* ascorbic acid, *GP* β-glycerophosphate

HA to modify polyphosphazene-based SMS. These modified composite SMSs are finalized via solvent sintering and exhibited good affinity for osteoblastic cell adhesion, proliferation, and function (9).

2) Biomineralization is an alternative route to introduce HA into PLGA matrix. Jabbarzadeh *et al.* (17) have modified PLGA SMSs via a biomineralization method where PLGA SMSs are surface-hydrolyzed with alkali liquor and then incubated in a modified simulated body fluid (mSBF) solution to induce apatite deposition on the surface of PLGA SMSs. The apatite layer on PLGA SMSs also results in protein absorption and timely delayed release.
3) Cushnie *et al.* (18) have developed a novel method to combine amorphous HA in PLGA SMSs *in situ* by mixing HA-phosphate-sourcing reagent and HA-calcium-sourcing reagent with PLGA matrix.

The advantage of the first method) is that the surface degradation of polymer matrix does not influence the bioactivity of SMSs. In contrast, bioactivity of SMSs produced via the second method) remarkably decreases after the degradation of HA-containing polymeric matrix on SMS surface.

Bioactive glass (BG) is a Ca-P-Si-O-based glass-ceramic biomaterial, which forms strong bonds with native bones *in vivo* due to the formation of apatite layer on interface (19). Yao *et al.* (20) have investigated synthetic conditions and kinetics of apatite formation on the surface of PLGA/BG SMSs. PLGA SMSs with 30 wt% BG show high biomineralizing tendencies *in vitro* and also favour osteogenic differentiation of mesenchymal stem cells (MSCs). Lu *et al.* (21) find that PLGA/BG SMSs possess superior mechanical properties and pose supportive to the adhesion, growth, and osteogenesis of osteoblastic cells *in vitro* in comparison with cases on pure PLGA SMSs. Mesoporous silica has also been used as filler to improve the mechanics, drug release, as well as cell affinity of PLGA SMSs. Shi *et al.* have evaluated these properties of mesoporous silica-HA (MS-HA) particle-modified PLGA SMSs. They demonstrate excellent apatite deposition when immersed in SBF solution for 7 days. The associated GS release period (from PLGA/MS-HA SMSs) reaches nearly 1 month (22). Additionally, the incorporation of MS-HA into PLGA SMSs also improves their cyto-compatibility. Porcine MSCs demonstrate higher viability and better proliferative profile on PLGA/MS-HA SMSs after 14 days of culture (22).

To date, SMSs have been mainly applied on bone and cartilage repair and regeneration. As one of the crucial requirements, scaffolds for bone repair must have appro-

priate porosity to allow the ingrowth of blood vessels. Human endothelial cells, which play a key role in angiogenesis and vasculogenesis, have been cultured on PLGA SMSs (24). The results indicate that SMSs are supportive for proliferation and commitment of endothelial cells. SMS-based *in vivo* trials for bone regeneration have also been investigated. Kofron *et al.* (25) have evaluated long-term osteoconductivity and degradation of PLGA SMSs in rabbit ulnar defective model. The results indicate that amorphous PLGA-based tubular SMSs have more mineralized bone tissue formation in comparison with semi-crystalline-based PLGA SMSs.

PLGA-based SMSs have been further endowed with deliverable drugs of alendronate (AL), dexamethasone (Dex), AL/ Dex, and Dex/ascorbic acid (AA) / β-glycerophosphate (GP) to facilitate osteogenesis of MSCs *in vitro* and *in vivo* (26,27). *In vivo* trials have been conducted with MSC-laden scaffolds in back subcutis of nude mice as well as femur defects on rabbit. SMSs have also been applied for fibrous cartilage tissue engineering. Growth factors such as transforming growth factor-beta1 (TGF-β1) and insulin-like growth factor-I (IGF-I) have been loaded in PLGA SMSs for potential fibrous cartilage repair applications (23).

In conclusion, given the superiority in mechanical properties and talents in controlled release and cell delivery, SMSs are a very competent candidate scaffolding model as efficacious substitutes for hard tissue reconstruction. Despite all these merits, there are still some limitations with SMSs for their applications, such as lower porosity (between 20% and 40%) (10). To this end, Wang *et al.* have adopted a novel strategy to fabricate SMSs with higher porosity. By using porous microspheres instead of plain microspheres, higher porosity of SMSs has been achieved (around 90%), but the mechanical properties are remarkably compromised (28). More studies are still pending for achieving further practical excellence with SMS systems in the near future.

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