Chapter 3
Injectable Calcium Phosphate Cements for Hard Tissue Repair

Fangping Chen, Yuanman Yu, Xiaoyu Ma, and Changsheng Liu

Abstract Injectable self-setting biomaterials are conducive to repair the complex-shaped bone voids by an invasive technique. With the combination of good injectability, fast setting in situ, easy shaping to complicated geometries, and excellent biocompatibility, injectable calcium phosphate cement (ICPC) has become an urgent need to repair the hard tissue. This chapter reviews the history and development, classification, properties (including injectability, rheological properties, fast setting, anti-washout, radiopacity, suspension stability, sealability), and their influence factors, as well as the applications of ICPC in hard tissue repair.

Keywords Injectable calcium phosphate cement • Minimally invasive treatment • Root canal filling • Sealability • Bone repairing

3.1 Introduction

The large number of orthopedic procedures performed each year has led to great interest in injectable biodegradable materials for bone regeneration. Osteoporosis is one of the three major diseases in the elderly, of which the incidence increases sharply year by year. It is estimated that over 100 million people in China suffer from osteoporosis, and the number of patients is expected to more than 200 million by 2050. Of these, the incidence of women and men over the age of 60 were 68.9% and 33% in year 2015, respectively. Additionally, high morbidity and mortality due to the increase of accidents and sports injuries bring a heavy burden to individuals and society. According to the latest statistics of the World Health Organization,

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dental diseases have been classified as the third major disease after cancer and cerebrovascular disease. The prevalence of caries in deciduous teeth of Chinese 5-year-old children was 66%, and 98.4% for 65–74 years old. Root canal therapy is currently the world's most common treatment of"pulpitis and apical periodontitis." Therefore, suitable biomaterials have become in great need for hard tissue repairing.

The repair of hard tissue defects is always an intractable problem perplexing surgeons and patients. Owing to their biological and physiochemical similarities to human bone and teeth, calcium phosphate cements (CPCs) have been extensively used in hard tissue repair and replacement. The biomaterials for replacement are available as powders, granules, or blocks. However, these forms are of limited effectiveness when cavities are not easily accessible. Some fractures in orthopedics, such as in distal radius, tibial plateau, and vertebral compression, only need to be treated by percutaneous injection without open surgery. In addition, nearly 60% of root canal filling materials causes the failure of endodontic surgery, especially for some small narrow and spindly apical foramens, clearances between root canal walls, and lateral accessory root canal. Therefore, injectable biomaterials are excellent candidates for bone grafting treatments in orthopedic and dental clinical.

Currently, the most commonly used injectable bone cement is poly(methyl methacrylate) (PMMA). Because of low viscosity and good injectability, PMMA can be applied to improve vertebral intensity and rigidity. Nevertheless, abundant clinic evidences show that there still remain many disadvantages including strong heat release, toxic monomers, and nondegradability when PMMA is applied in percutaneous vertebroplasty. For these reasons, there are views against applications of PMMA for vertebra only if it is used for palliative care of vertebral metastatic tumors.

With the combination of good injectability, fast setting in situ, easy shaping to complicated geometries, and excellent biocompatibility, injectable calcium phosphate cement (ICPC) has become an urgent need to repair the hard tissue. In addition, the strengthening and the stabilization of an osteoporotic bone tissue can be carried out solely by injection without excision or destruction of the residual bone matrix. Injectable cement has been attempted to use in paste form to minimize the invasiveness of surgical procedures and reduce the site of scars and postoperative pain. This chapter will provide an overview of injectable calcium phosphate cement for use in bone and teeth regeneration.

3.2 History and Development of ICPC

Injectable biphasic calcium phosphate (BCP) bone cement was first studied by Frenchman Daculsi et al. in 1996 [1]. The cement was easily injectable with the addition of a 2% methyl cellulose carrier gel in BCP particles (60/40 HA/β-TCP weight ratio). After 10 weeks implantation in rabbit distal femurs, bone ingrowth proceeded from the perimeter inward at a greater rate than in BCP blocks alone [2]. However, the low initial mechanical properties of BCP directly lead to difficulty in maintenance of the composite within the defect during surgery.

Since 1997, many kinds of injectable cements have been investigated. Knaack et al. [3] studied injectable calcium phosphate bone substitute(ABS) (a-BSM™, ETEX Corporation, Cambridge, MA), which was prepared from calcium phosphate precursor powders with an unbuffered physiological saline solution. The result showed that the autograft and ABS were associated with similar new bone growth and defect filling characteristics, and materials were nearly absorbed after implantation for 26 weeks.

ChronOS Inject (Synthes AG, Switzerland) consists of a powder mixture of brushite-based calcium phosphate and a sodium hyaluronate solution. The chronOS Inject as bone void filler was injected in fresh fractures of follow-up for 1 year. High patient satisfaction (92%) with treatment was achieved, despite the loss of reduction being described in 11% of proximal tibia and 2% of distal radius fractures. Overall, the material showed good outcome in the majority of patients and adequate resorption characteristics. However, stable internal fixation and sufficient bone quality are essential requirements for chronOS Inject [4].

Matsumine et al. [5] injected ICPC after bone excision and carried out X-ray tracing in 56 patients, showing that ICPC was adaptable to surrounding tissue. The HA and ß-calcium phosphate complex was developed as an injectable, bone marrow-containing, and two-phase bone repair material with improved osteoconductive property. In vivo histological studies have also demonstrated that nanocrystal HA has better osteogenic property and vascular regeneration performance [6].

Our group developed CPC, composed of TECP and DCPA, as an injectable biomaterial (Injectable CaP, Rebone®) in 1998. Injectable CaP was firstly used in percutaneous vertebroplasty in 2003 [7–9]. Then the biodegradable injectable CaP was scaled up for wide clinic applications, and now it has been widely used for more than 20,000 cases in 300 hospitals. Clinical studies have shown that the injectable CaP can enhance vertebral body strength and stiffness, restore spinal function characteristics, and at the same time significantly reduce the potential serious side effects such as intraoperative blood pressure drop, pulmonary embolism, spinal cord and nerve root injury, etc.

Zhu XS and Yang HL [10] compared the properties of Injectable CaP, CaS (calcium sulfate), and PMMA by injecting them into the defect vertebrae L2–L5 in 24 adult female sheep. Animals were sacrificed after 2, 12, and 24 weeks of the bone filler augmentation, respectively. The vertebrae of L2–L6 were collected, and their biomechanical strength/stiffness, osseointegration activity, and biodegradability were evaluated. At all three time points tested, the PMMA-augmented lumbar vertebra had the highest biomechanical strength and stiffness, followed by the intact vertebra L6. Injectable CaP (Rebone®) and CaS significantly improved the strength, but did not yet restore it to the normal level. Osteogenesis occurred in the injectable CaP (Rebone®)-augmented defect vertebrae at 12 and 24 weeks. The result indicated that injectable CaP (Rebone®) and CaS were effective enough to strengthen the fractured lumbar vertebrae in a time-dependent manner [10]. Although these materials were reported to have good compatibility and excellent injectability, initial mechanical properties were still low to support the tissue growth. Xu [11] studied the clinical effect of thoracic and lumbar fractures treated by pedicle screw

fixation and vertebroplasty with calcium phosphate cement with the help of C-shaped arm X-ray machine. The result showed that all the 21 cases were treated successfully without postoperative infection, neurologic symptoms, and internal fixation complications. During the period of follow-up, outcomes were satisfactory and no injured vertebral lost its height obviously. Furthermore, Xu found that the vertebroplasty treated by injectable calcium phosphate cement contributed to the reconstruction of the injured vertebra, and the biomechanics of postoperative vertebral body restored to the level before fracture [12].

SRS (skeletal repair system; Norian Company, USA) is a typical injectable calcium phosphate bone cement, mainly composed of TCP, calcium phosphate, calcium carbonate, and sodium phosphate. To inject the cement into the porous structure easily, an injection gun was applied especially in the osteoporotic vertebral body. Carbonated hydroxyapatite was formed after setting for 10 min. The result showed that SRS bound closely with surrounding bone, with compressive strength of 10 MPa and up to 55 MPa after 12 h. Serraj Siham et al. [13] injected Norian SRS in the subchondral bone defect. 15.3% of reduction of the fracture required revision surgery after surgery for 8 weeks due to partial bone loss. All other patients had complications, and fractures were healed without any displacement. The high mechanical strength of the cement allowed early weight bearing after a mean postoperative period of 4.5 weeks.

Many existing calcium phosphate materials degrade very slowly and have low strength, leading to the decreased bone regeneration at the site of the implant [14]. In an effort to address these concerns, researchers have chosen to investigate polymeric materials for use. Examples of naturally occurring biopolymers used include chitosan, alginic acid and hyaluronic acid, polyesters, and PMMA. The frangibility and mechanical property of calcium hydrogen phosphate were significantly improved by adding PMMA molecules. Compared to macroporous biphasic calcium phosphate, the composites of hydroxypropyl methyl cellulose and calcium phosphate as injectable bone materials had improved biological activity at the early implantation stage. The poly(propylene fumarate) and calcium phosphate composites can be injectably used for the treatment of femoral head necrosis. The results showed that the mechanical properties of the composites were enhanced with the increase of calcium phosphate content in the composites [15, 16].

Some elements or components can improve the bone repair ability of the ICPC. The CPC containing strontium carbonate had better injectability and compressive strength, which had an effect on the distribution of pores in the cement [17]. The mixture of calcium phosphate and calcium sulfate had not only good injectability, appropriate setting time, and mechanical property but also improved degradability and osteogenetic ability [18]. Studies found that after hydration, biphasic bone substitute materials mixed by calcium sulfate and tricalcium phosphate could form calcium sulfate dihydrate and decalcified hydroxyapatite simultaneously [19]. The mechanical strength of composite IBS matches the human cancellous bone. Therefore, it is suitable for the treatment of the vertebral body and cystic cavities.

In addition to SRS, commercially available injectable bone cements are shown in Table 3.1.

Table 3.1 Examples of commercially available injectable bone cements

	Company	Commercially available product	Composition	Commercially available forms	Current proof
Bone repair	AlloSource	AlloFuse™	Heat-sensitive copolymer with DBM	Injectable gel and putty	Case reports
					Animal studies
					Cell culture
	Biomet Osteobiologics	BonePlast®	Calcium sulfate with or without HA/CC composite granules	Various volumes of powder and setting solution	Case reports
					Animal studies
		BonePlast® quick set	Calcium sulfate	Quick-setting paste	Case reports
					Animal studies
	Exactech	Optefil®	DBM suspended in gelatin carrier	Injectable bone paste dry powder ready to be hydrated	Human studies
					Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction
		Opteform®	DBM and cortical cancellous chips suspended in gelatin carrier	Formable putty or dry powder ready to be hydrated	Human studies
					Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction
	Integra Orthobiologics/ IsoTis OrthoBiologics	Accell Connexus®	DBM, accell bone matrix, reverse phase medium	Injectable putty	Human studies
					Case reports
					Animal studies
					Every DBM lot tested for osteoinduction
		Accell Evo3™	DBM, accell bone matrix, reverse phase medium	Injectable putty	Animal studies
					Every DBM lot tested for osteoinduction
		DynaGraft II	DBM, reverse phase medium	Injectable putty	Human studies
					Case reports
					Animal studies
					Every DBM lot tested for osteoinduction

(continued)

Table 3.1 (continued)

	Company	Commercially available product	Composition	Commercially available forms	Current proof
		OrthoBlast II	DBM, cancellous bone, reverse phase medium	Injectable putty	Human studies
					Case reports
					Animal studies
					Every DBM lot tested for osteoinduction
		Integra Mozaik™	80% highly purified b-TCP/20% highly purified type 1 collagen	Strip and putty	Human studies
					Case reports
					Animal studies
	LifeNet Health	Optium DBM®	DBM combined with glycerol carrier	Formable putty (bone fibers) and injectable gel (bone particles)	Human studies
					Case reports
					Animal studies
	Medtronic Spinal & Biologics	MasterGraft® putty	Biphasic calcium phosphate and collagen	Moldable putty	Animal studies
		Osteofil® DBM	DBM in porcine gelatin	Injectable paste and moldable strips	Animal studies
					Case reports
		Progenix™ plus	DBM in type 1 bovine collagen and sodium alginate	Putty with demineralized cortical bone chips	Animal studies
					Case reports
		Progenix™ putty	DBM in type 1 bovine collagen and sodium alginate	Ready to use injectable putty	Animal studies
					Case reports
	MTF/Synthes	DBX®	DBM in sodium hyaluronate carrier	Paste, putty mix and strip	Human studies
					Case reports
					Animal studies
	Orthovita	Vitoss®	100% β-TCP and 80% β-TCP/20% collagen and 70% β-TCP/20% collagen/10% bioactive glass	Putty, strip, flow, morsels and shapes	Published human studies (level I and III)
					Case reports
					Animal studies

(continued)

Table 3.1 (continued)

	Company	Commercially available product	Composition	Commercially available forms	Current proof
	Osteotech	Grafton®	DBM fibers with demineralized cortical cubes	Packable graft	Peer-reviewed published human studies (incl. level I–II prospective studies)
		Crunch®			Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction
		Grafton® gel	DBM in a syringe	MIS and percutaneous injectable graft	Peer-reviewed published human studies (incl. level I–II prospective studies)
					Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction
		Grafton® orthoblend large defect	DBM fibers with crushed cancellous chips	Packable graft	Peer-reviewed published human studies (incl. level I–II prospective studies)
					Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction
		Grafton® orthoblend small defect	DBM fibers with larger cancellous chips	Packable moldable graft	Peer-reviewed published human studies (incl. level I–II prospective studies)
					Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction

(continued)

Table 3.1 (continued)

	Company	Commercially available product	Composition	Commercially available forms	Current proof
	Osteotech	Grafton Plus® paste	DBM in a syringe	Injectable MIS graft, resists irrigation	Peer-reviewed published human studies (incl. level I–II prospective studies)
					Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction
		Grafton® putty	DBM fiber technology	Packable moldable graft	Peer-reviewed published human studies (incl. level I–II prospective studies)
					Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction
	Regeneration Technologies	BioSet™	DBM combined with natural gelatin carrier	Injectable paste, injectable putty, strips and blocks with cortical cancellous chips	Human studies
					Case reports
					Animal studies
					Every lot tested in vivo for osteoinduction
	Smith & Nephew	Viagraf	DBM combined with glycerol	Putty, paste, gel, crunch and flex	Animal studies
	Synthes	Norian® SRS®	Calcium phosphate	Injectable paste	Human studies
					Case reports
					Animal studies
		Norian® SRS® fast set putty	Calcium phosphate	Moldable putty	Human studies
					Case reports
					Animal studies

(continued)

Table 3.1 (continued)

	Company	Commercially available product	Composition	Commercially available forms	Current proof
	Wright Medical Technology	Allomatrix®	DBM with/ without CBM in surgical grade calcium sulfate powder	Various volumes of injectable/ formable putty	Human studies Case reports Animal studies Cell culture
		Allomatrix® RCS	DBM with Caciplex™ technology in surgical grade calcium sulfate powder	Various volumes of formable putty	Animal studies
		MIIG® X3	High strength surgical grade calcium sulfate	Minimally invasive injectable graft for compression fractures	Human studies Case reports Animal studies
		PRO-DENSE® injectable regenerative graft	75% calcium sulfate and 25% calcium phosphate	Procedure kits, various volumes of injectable paste	Human studies Case reports Animal studies
		PRO-STIM™ injectable inductive graft	50% calcium sulfate, 10% calcium phosphate, and 40% DBM by weight	Procedure kits, various volumes of injectable paste/formable putty	Case reports Animal studies
	Zimmer	CopiOs® bone void filler	Dibasic calcium phosphate and type 1 collagen	Sponge and paste	Case reports Animal studies
		Puros® DBM	Allograft DBM putty (putty with chips includes allograft chips from the same donor)	Putty and putty with chips	Every lot tested in an in vivo rat assay for osteoinductive potential demonstrating bone formation in an ectopic model

(continued)

Table 3.1 (continued)

	Company	Commercially available product	Composition	Commercially available forms	Current proof
Root canal	Morita	Vitapex	30% calcium hydroxide, 40.4% triiodomethane, 22.4% silicone oil, 6.9% filler	Alone (temporarily) or in conjunction with the Gutta Perch (permanent). Paste	
	Meta	Metapex	Barium sulfate, calcium hydroxide	Exposed pulp of pulp capping and pulpotomy	
				Root canal leakage	
				Apexification	
				Hard tissue barrier formation	
				Root canal filling material	
	Rebone	ReboneGutai™	Superfine calcium phosphate paste	Sponge and paste	Human studies
				Bone defect repair and root canal filling	Case reports
					Animal studies
	PD	Pro-Pul-Pan	Powder: zinc oxide 9.0%, thymol iodine 22.5% solution: eugenol	Root canal filling paste	

A. CaP calcium phosphate, *DCPD* dicalcium phosphate dihydrate, *TCP* tricalcium phosphate, *TTCP* tetracalcium phosphate, *HA* hydroxyapatite, *DCP* dicalcium phosphate, *MCPM* monocalcium phosphate monohydrate, *Bis-GMA* 2,2-bis[4-(2-hydroxymehacryloxypropyl) phenyl] propane, *Bis-EMA* 2,2-bis[4-(2-methacryloxyethoxy)]phenyl propane, *TEGDMA* triethylene glycol dimethacrylate, *3MPS* 3-methylacryloxy-propyltrimethoxysilane, *BPO* benzoyl peroxide, *DHEPT* di(hydroxy-ethyl)-p-toluidine

3.3 Classification of ICPC

Ideally, ICPC can be injected into the damaged tissues and then mold to the shape of the bone cavity in situ filling in the defects. Before injection, the ICPC should remain stable, be easy to inject through the percutaneous or small bone window, and set rapidly after implantation in defects.

Conventional injectable CPC, taking a variety of aqueous media (e.g., distilled water, phosphate-buffered saline solution, sodium phosphate, citrate buffer solution, carbonate buffer solution, or saline solution) as setting liquid, has proven to be an alternative bone substitute [20]. The aqueous phase ICPC has combined characteristics of biocompatibility, osteoconductivity, fast solidifying, high initial mechanical strength, and easy shaping for any complicated contours of bone defects [21].

However, several drawbacks of ICPC prevent them from gaining universal acceptance. It is worthwhile to note that the aqueous phase ICPC has to be prepared just before implantation, since setting starts from the moment that the powder comes in contact with water. Clearly, it is difficult for the clinician to mix the powder with liquid thoroughly and hard to inject the aqueous phase ICPC into the defect within a prescribed time as well. In addition, the operation of on-site powder-liquid mixing prolongs the surgical time. It may degrade physical and chemical properties of ICPC and even cause potential side effects on tissue repairing because of inhomogeneous mixing and insufficient filling stemming from the limited mixing time [22]. In order to overcome herein before shortages, ICPCs can be prepared as being aqueous injectable calcium phosphate cement (a-ICPC) or nonaqueous injectable calcium phosphate cement (n-ICPC) on the basis of the nature of the liquid phase.

Takagi and Xu [23] made nonaqueous phase setting liquid from nontoxic organic liquids which were immiscible with water, such as glycerol, polypropylene glycol, and low molecular weight polyethylene glycol (PEG) liquid. The n-ICPC can be mixed in advance in a controlled environment to keep the system anhydrous. It avoids the temporary mixture of solid-liquid two-phase to shorten the operation time and shows the characteristic of long-term preservation.

Premixed acidic CPC was formed by mixing the powder phases with glycerol as a delivery system for water-solubilized simvastatin. The lower doses of SVA showed an approximately fourfold increase in mineralization as compared to the control. The results also demonstrated that premixed acidic CPC is a good option for local delivery of SVA leading to a prolonged stimulation of osteogenesis [24]. The cements with smaller particle sizes showed higher compressive strength and more difficulty to inject. The addition of granules made the cements easier to inject, but prolonged the setting time and reduced the strengths. Therefore, the particle size can be used to control the handling and physical properties of premixed cements [25].

Our group has developed the n-ICPC root canal paste by well-premixed ultrafine calcium phosphate powders with nonaqueous phase organic setting liquid since the year of 2003. n-ICPC has a series of excellent properties including good injectability, self-setting, excellent biocompatibility, controllable degradation, and proper compressive strength. Moreover, the paste can seal root canal effectively and be compatible with apical surrounding tissues without root canal filling reaction. The success rate of surgery with n-ICPC in clinic applications reached over 98.6%. More importantly, it did not need gutta-percha point to close the root canal, indicating the great potential of ICPC in root canal treatment.

3.4 Properties of ICPC

The ultimate goal of injectable calcium phosphate cement is to offer an ideal bone substitute for better clinic performance. Nowadays, the main disadvantages of ICPC are:

1. Anti-washout property of ICPC is so poor that the slurry is easily dispersed by blood or tissue fluid.

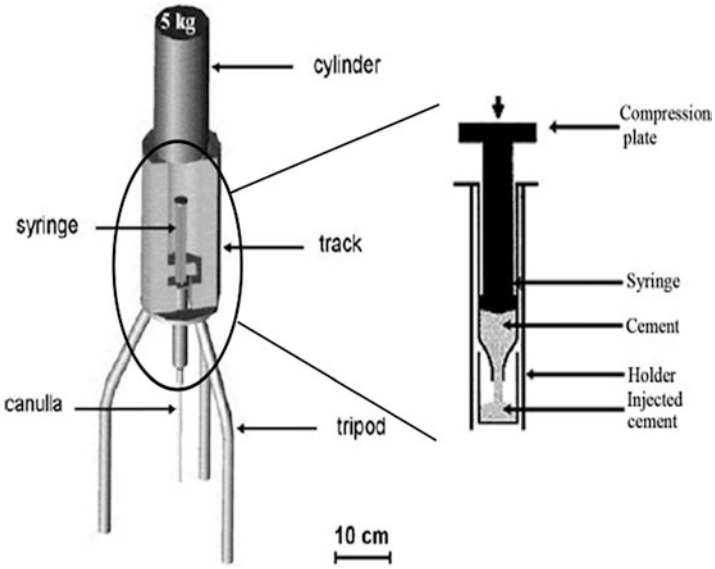

Fig. 3.1 Devices for evaluating the injectability of paste

2. Poor injectability for some cements and solid-liquid separation issue.
3. Contradiction between injectability and setting time still remains. ICPC with good injectability has long setting time, while ICPC with short setting time is difficult to inject.

Therefore, the improvement of the biomaterial properties or the development of related products should obey the clinical demands. ICPC should satisfy the required properties as follows.

3.4.1 Injectability

Injectable cement not only has the potential to fill the lesions and stabilizes the osteoporotic bone at risk for fracture but also conforms to the defect area such as periodontal bone repair and tooth root canal fillings. Injectability is related to the ability of a paste to extrude through a syringe or to be filled into the bone defects under pressure keeping the homogeneity. It is generally measured by correlating either to the force required for complete extrusion or to the quantity of the cement extruded during a certain period. Different techniques and parameters were applied to measure and quantify injectability of bone cements. However, there is no unified standard for measuring injectability. Generally speaking, injectability is measured by the percentage of a paste that could be injected from a syringe under a constant compressive load. Figure 3.1 shows the device for evaluating the injectability used in our group.

The injectability of paste is affected by many factors, such as the size and shape of particles, the ratio of solid phase to liquid phase, the additives and injection force, etc. It has been reported that small crystals favored a good injectability of α-BSM®. Although a larger amount of liquid was required to form a paste with small particles, injectability and cohesion of α-BSM were generally very good [26, 27]. Ishikawa found that the cements with round particles, regular shape, and higher ratio of liquid to powder could be injected easily.

The lack of inherent viscosity limits the fabrication of injectable cements. When injected from a delivery system, the pastes will undergo "filter pressing" or phase separation, in which the liquid part gets expelled leaving behind the solid phase [28]. An effective method to break through the bottleneck is to incorporate gelling agents or viscous media. The choice, however, is limited because of the requirement of biocompatibility, as the complex interaction between the ions of the additives may lead to the formation of toxic moieties. Moreover, the additives can adversely affect the setting and mechanical properties of the cement. Typically, ICPCs with good injectability have been successfully fabricated with the use of low quantities of glycerol phosphate, chitosan, alginate, cellulose, glycerol, lactic acid, citric acid, etc., which have reasonable biocompatibility. These additives not only decreased the interactions between particles or increased the viscidity of liquid phase but also prevent the phase separation between the solid and liquid. Our experiments have demonstrated that the white dextrin, Xanthan gum, and PEG-6000 promoted the injectability of ICPC to a certain extent [29, 30].

When the paste was injected into the bone defects, the injection resistance would increase due to the pressure inside the body cavity. Therefore, the injectable cement was propelled by motor pushers. Obviously, the better the injectability of ICPC is, the smaller the propelling force or the injection pressure will be. Moreover, the parameters of the injection device also affect the injectability. Namely, shorter cannulas with a larger diameter, as well as smaller injection rates were found to contribute to a better injectability. More importantly, the injectability is time dependent, which decreases with the advance of the hydration of CPC. That is the reason that injectability should be calculated from the starting point of mixing the solid with the liquid.

3.4.2 Rheological Properties

During the whole injection process, the flow characteristics of ICPC are quite important. The slurry should be injectable with low resistance, and there is no powder-liquid phase separation. These requirements mainly depend on a suitable rheological property of ICPC. In this sense, rheological parameters are suitable to quantitatively describe the flow or injection process. In such a reactive system of ICPC, the internal microstructure changes with the time. These changes affect not only the flow and workability but also the mechanical properties of the cement after hydration. Therefore, the investigation of the rheological properties of ICPC would be beneficial to improve the performance characteristics of the ICPC slurry.

Table 3.2 Thixotropy loop area and stress slowdown rate of ICPC with different PEG-6000 contents

Content (wt%)	A_τ(Pa s^{-1})	$\Delta\tau$(%)
ICPC (0)	5100.2	15.61
ICPC (0.5%)	5998.7	9.54
ICPC (1%)	6521.0	8.78
ICPC (5%)	8860.4	11.70
ICPC (10%)	1.208E+04	11.74
ICPC (20%)	1.479E+04	12.61

Rheologies include the steady rheology and dynamic rheology. The steady rheology is to measure the shear stresses under consecutive linear increasing and decreasing in the rotational shear rates. Viscosity is an indicator of a fluidic resistance to flow, describing internal friction in a moving fluid. Low viscosity favors the injectability of cement. Yield stress is the critical strength that must be applied to a material allowing it to flow. The bigger the yield stress, the more difficult a cement is to inject. Thixotropy, a phenomenon in steady rheology, is defined as the continuous decrease of apparent viscosity with the time under shear and subsequent recovery of viscosity when the flow is discontinued. This parameter reflects the complex interaction forces of particles and the formation of isolated large flocs or single particulate structure throughout the whole materials.

Thixotropic hysteresis loop area was used to evaluate the magnitude of thixotropy of the paste during motion, which is proportional to the energy required to break down the thixotropic structure. The larger the thixotropic hysteresis loop area, the more energy is required to break down the thixotropic structure. Liu et al. explored the steady rheological properties in ICPC reactive system. The results indicated that the yield stress and the area of the thixotropic hysteresis loop were enlarged as the setting process progressed. With the increase of the ratio of solid to liquid, structural strength, viscosity within the ICPC improved, and the corresponding thixotropy became larger. It also revealed the microstructure development of ICPC during the whole setting process, and the factors affected the rheological properties [31]. Chen reported that the change of the hysteresis loop area and stress slowdown rate with the increased PEG-6000 content (Table 3.2). The degree of thixotropy is judged from the area between the up and down curves. By adding PEG-6000 to the cement liquid, the thixotropy loop area of the pastes notably increased, thus enhance the stability of ICPC paste. Among these ICPC pastes, the ICPC (20%) is with the highest thixotropy, and the pure ICPC paste is least in thixotropy. In addition, the addition of PEG-6000 reduced the $\Delta\tau$ of the ICPC pastes till 1 wt%. But above 1 wt%, the $\Delta\tau$ would keep almost constant. It is also clear that the stability of the ICPC paste can be improved by introducing proper content of PEG-6000 (Fig. 3.2).

Dynamic rheology is the dynamic mechanical oscillatory strain applied to a sample, allowing the sample to be subjected to a strain and viscous and elastic properties of the sample and to be measured simultaneously to explore the internal structure

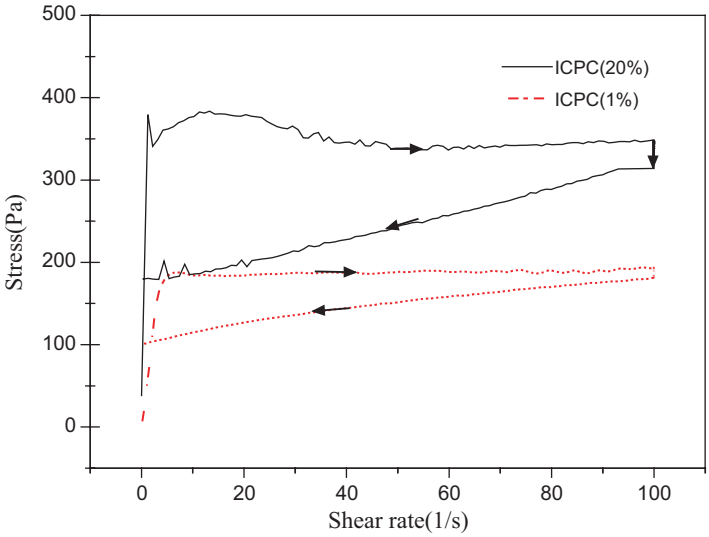

Fig. 3.2 Thixotropy loop curves of ICPC pastes (Reproduced from Ref. [29] by permission. Copyright © 2010 Elsevier B.V)

of the sample. The recovery of the material after deformation was characterized by the storage modulus G′ and loss modulus G″. G′ represents the ability of the material to store the energy of deformation, while G″ refers to the ability of materials to consume the energy of deformation. Figure 3.3 showed that all the storage modulus G′, loss modulus G″ and complex viscosity η* increased with hydration time, further verifying that the recovery of ICPC after deformation was involved with the components of solids materials [29].

Rheological properties are related to the particle size, distribution of the components, the ratio of powder to liquid, and the surface charge of the particles in contact with the liquid phase [32]. Gbureck et al. improved the injectability of activated α-tricalcium phosphate (α-TCP)-based cement by adding several fine-particle-sized fillers and showed that the introduction of inert filler reduced the viscosity significantly [33]. Baroud et al. found that P/L, milling time, and additives significantly affected the rheological characterization of concentrated aqueous β-TCP suspensions, such as the viscosity and yield stress [34].

To make ICPC easy to operate in the clinic, some rheological additives are added to improve the thixotropy and the stability of the slurry, the fluidity, water resistance, the coagulation time, and so on. The effect of lactic acid, glycerol, acetyl chitin, glycerin, and sodium phosphate on the ICPC was studied, but the comprehensive performance was still not satisfied [35]. Jin et al. [36] investigated the influence of polyvinylpyrrolidone (PVP) on the rheological characteristics of ICPC through an advanced rheometric expansion system (ARES). By introducing the PVP with different value of K and different quantity into the setting liquid, the rheological behaviors of CPC slurry could be improved greatly. The results showed that

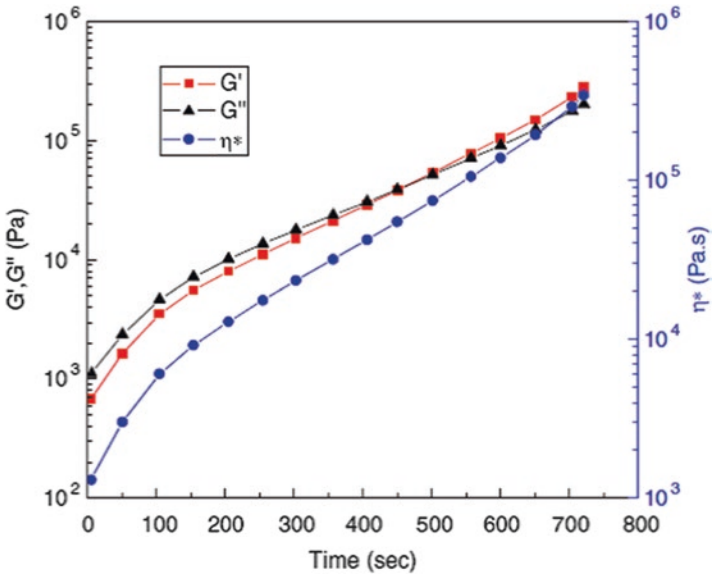

Fig. 3.3 Time-sweep curves of CPC slurry at 1 min after mixing. Stain, 1%; ω, 1 rad s^{-1}; P/L ratio, 2.0 (Reproduced from Ref. [31] by permission. Copyright © 2006 Elsevier B.V)

the CPC slurry was a typical non-Newtonian shear-thinning fluid, and the addition of PVP could not change this property of CPC slurry. But due to the existence of PVP, the viscosity of CPC slurry and the area of thixotropic loop were increased, and the restorability was also improved (Fig. 3.4), leading to the improved stability of CPC slurry. These results were also explained in Tables 3.3 and 3.4. The study showed that the 20% PVPK90 and 30% PVPK30 improved the rheological properties of ICPC slurry, which makes the ICPC more suitable as an injectable material.

In addition, the effect of water-soluble polymer PEG on ICPC was also investigated. Viscosity curves showed that the ICPC containing PEG had obvious shear-thinning behavior. With the increase of additive concentration, the viscosity of ICPC gradually increased. Curve slope remains unchanged, which illustrates different concentrations of additive had the same effect on the shear thinning of the system. Thixotropic loop curve showed that the ICPC is characterized with thixotropy. One percent PEG would not give much benefit on thixotropy of ICPC. Further increasing the PEG content up to 30% and 50%, the area of thixotropic loop significantly increased, and the thixotropy enhanced greatly. Therefore, the improvement of the structure strength of ICPC lies to the formation of network composed of particles and polymer with the interactions between PEG and water. Obviously, the thickening effect of PEG can increase the viscosity and the stability of the system (Fig. 3.5).

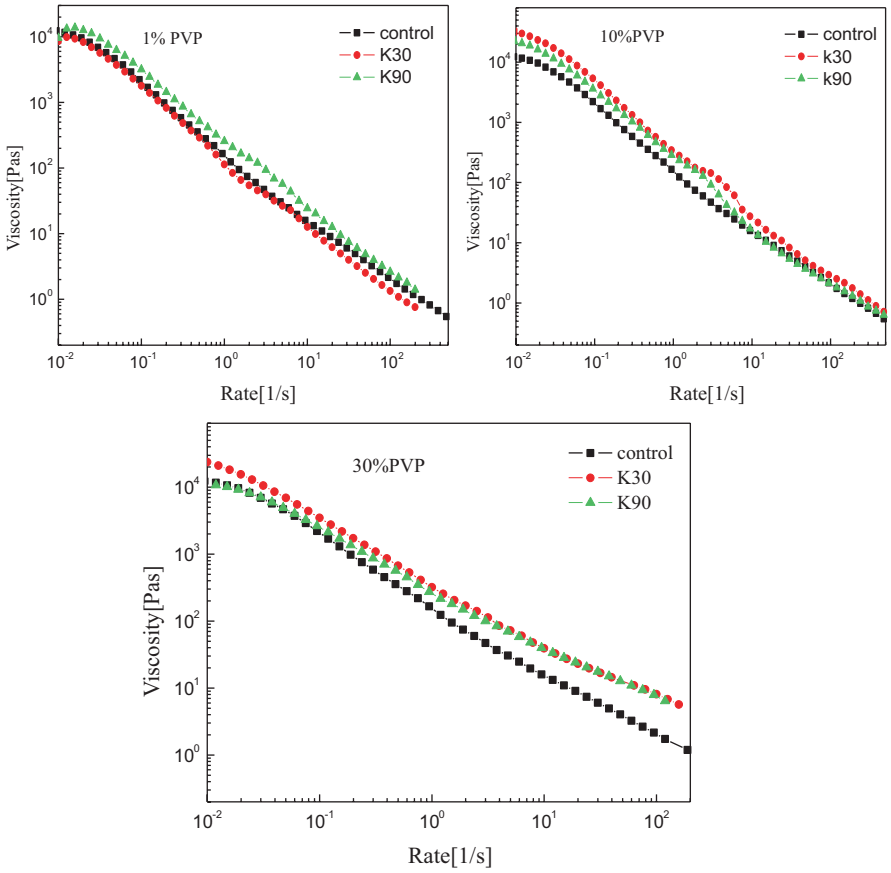

Fig. 3.4 Effect of K30 and K90 on viscosity curves of ICPC with 1%, 10%, and 30% PVP (Reproduced from Ref. [36] by permission)

Table 3.3 Results of thixotropic loop test of CPC slurry with PVP K30

Concentration/%	Area of loop	Degradation of strain/%
0 (control)	5100.2	15.61
1	6756.1	18.78
5	8297.9	12.61
10	13380.0	15.39
20	15440.0	15.30
30	21740.0	10.57

Reproduced from Ref. [36] by permission

Table 3.4 Results of thixotropic loop test of ICPC with PVP K90

Concentration/%	Area of loop	Degradation of strain/%
0 (control)	5100.2	15.61
1	7538.0	9.97
5	9250.2	12.57
10	11850.0	13.03
20	11320.0	9.78
30	19000.0	17.40

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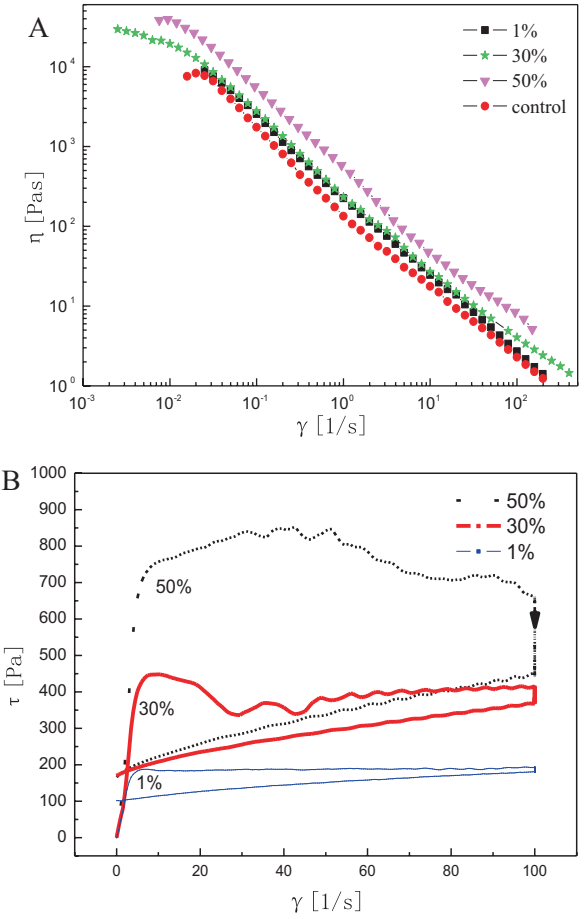

Fig. 3.5 Viscosity curves (**a**) and thixotropic loop (**b**) with PEG2000 (1%, 30%, 50%)

3.4.3 Fast Setting

A good injectability is an important property required for ICPC applications. Furthermore, ICPC must set slowly enough to provide sufficient time for a surgeon to perform implantation. However, it also required fast setting enough to prevent washout and delayed operation. Therefore, setting time should be in a suitable range. The environment of ICPC implanted will also affect the setting time. For instance, magnesium ions in blood along with many organic materials will inhibit the setting of ICPC and thus make the setting time longer in serum than in distilled water. In addition, if the liquid phase of nonaqueous ICPC needs to exchange with the water or tissue liquid, the setting time thus becomes longer than those of aqueous ICPC. Therefore, it is necessary to shorten the setting time for the nonaqueous ICPC.

The Gillmore needle test and Vicat needle test define the setting time according to such standards as ASTM C266-89 and ASTM C191-92. The initial (I) and final (F) setting time are determined using the time required for the penetration of the needle into a paste specimen of specific consistency to a specific depth. In particular, a light and thick needle is used to measure the initial setting time I, while a heavy and thin needle for the final setting time F. The clinical meaning is that the cement paste should be implanted before time I and that the wound can be closed after time F.

In recent years, nondestructive methods have been attempted to online monitor the setting process, e.g., using electrical resistivity, ultrasonic, isothermal differential scanning calorimetry, dynamic rheological test and alternating current (AC) impedance spectroscopy, Fourier-transform infrared spectroscopy, solid-state NMR, X-ray diffraction, and energy-dispersive X-ray diffraction. An ultrasound through transmission is a novel method to analyze the entire setting process in situ about the nonaqueous injectable cement (glycerol as setting liquid) changes in body fluids. The material constants including the Young's modulus (E), the Poisson ratio (ν), and the shear modulus (G) at each step of the set process were monitored, as well as for the comprehensive systematic tracking of changes in the cement paste during the setting and hardening process of the cement, starting from the moment of immersion of cement in body fluids to a final product in the form of a hardened cement, and even the subsequent degradation process [37]. According to the clinic experience, 5–15 min is the reasonable range for the setting of ICPC (Fig. 3.6). Longer setting time would be conducive to the doctor's operation, but longer setting than necessary tends to make ICPC crumble upon early contact with blood or other fluids. Therefore, it is necessary to regulate the setting time of ICPC in a proper range.

The setting time of ICPC was affected by many factors. Previous literature reported that setting time was significantly shortened by decreasing the particle size of TECP and DCPA or introduction of HA crystal seeds [38]. The dissolution of phosphate or calcium salts (e.g., Na_2HPO_4 and $CaCl_2$ solutions) increased the supersaturation of Ca^{2+} and PO_4^{3-} in the solution during hydration, thus improving the impetus of the reaction and accelerating the setting. Citric acid can provide pair

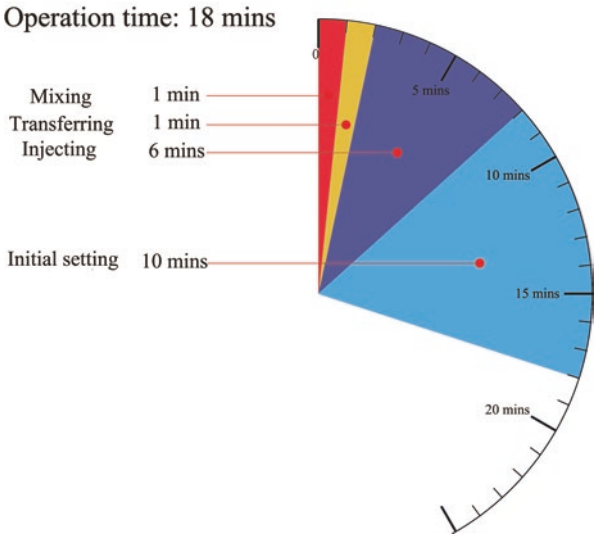

Fig. 3.6 The reasonable time range of ICPC

electrons and calcium ions have electron vacancies. Thus, citric acid can react with calcium in the solution and transform into a chelate compound through chelation, accelerating the setting of ICPC.

The shortened setting time with acceptable injectability provides an easy way for the surgeon to use such materials in some urgent cases. Related studies showed that the injectability and the setting time can be balanced, and ICPC with satisfied fluidity and injectability for clinical operation can be prepared by introducing the additives and regulating concentration, particle sizes, ratio of powder to liquid, and so on.

3.4.4 Anti-washout

Injectable cement washout can occur in vivo when it comes in contact with physiological fluids or when bleeding occurs due to difficulty in achieving complete hemostasis. Some cements would be washed out completely when immersed in simulated body fluid immediately after mixing. In percutaneous vertebroplasty, particles are ready to fall off the pastes and enter the cardiovascular system along with the blood, which often cause vascular blockage to thrombus, pulmonary embolism, or other complications. In this sense, it is necessary to improve the anti-washout property of the injectable cements.

Anti-washout property was usually estimated by qualitative analysis and quantitative analysis. Qualitative analysis of the washout resistance for injectable calcium phosphate cement relies mainly on the naked-eye observation, such as the disintegration of the pastes and the turbidity of the soaked liquid. Quantitative analysis

refers to calculate the washout mass loss rate of the paste during the period of immersion according to Eq. 3.1. Anti-washout property is affected by the hydration rate, the formation of waterproof membrane, the temperature of the simulated body fluid, and the speed of the shaker.

$$\text{Washout mass loss}\% = \frac{\text{Mass lost during immersion}}{\text{Total mass before immersion}} \times 100\% \tag{3.1}$$

The higher ratio of the liquid to solid and the prolonged setting time tend to make ICPCs crumble easily by plasma or other body fluids before the paste hardens. The bad resistance to water of ICPCs further prolongs the setting time. All these restrict the wide clinic applications of injectable cements. Thus, great efforts have been made to improve the washout resistance of ICPC.

Preventing the fluids from spreading to the cement paste is an effective strategy to improve the anti-washout property of injectable cement. To attain this goal, gelling agents have been utilized to impart viscosity to cement. Ishikawa [39] revealed that sodium alginate in the liquid could prevent the injectable cements from collapse and could be set normally. The reason is that calcium ions can react with sodium alginate to form insoluble calcium alginate hydrogel, which protect the CPC from being washed out. Takechi [40] showed that carboxymethyl cellulose, chitosan acetate, and chitosan lactate improved the operation performances of injectable cements, but prolonged the setting time. Although the detailed mechanisms of washout resistance for injectable cements with gelling agents have not been fully clarified at present, the improvement in anti-washout properties can be attributed to the adhesive property and negative charge of gel, which serves as a "glue" to fuse the particles together. Wang evaluated the water resistance, setting time, compressive strength, and biocompatibility of ICPC with cellulose as additives [41]. In addition, modified starch [42], white dextrin, and locust bean gum [43] have been tried to prepare the anti-washout type cement. Inositol phosphate was added to improve the anti-washout properties by virtue of the strong chelating capability to calcium ions in novel CPC system [44]. The development of versatile additives improves the anti-washout performance, but they may deteriorate other properties of injectable cements.

Speeding up the setting process of ICPC is another alternative way to improve the anti-washout property. An amount of wollastonite promoted the hydration reaction and shortened the setting time of ICPC, thus improving the anti-washout. The amorphous silica layer was formed by the wollastonite dissolution and absorbed on the surface of the CPC particles, which keeps the paste from being eroded by liquid [45]. Magnesium phosphate cement (MPC), consisting of magnesium oxide (MgO) and calcium biphosphate (Ca $(H_2PO_4)_2$), is of special interest due to its rapid setting and high initial mechanical strength in comparison with the phosphate cement [46]. The major components of MPC could react in aqueous environment to form magnesium phosphate ($Mg_3(PO_4)_2$) and calcium triphosphate ($Ca_3(PO_4)_2$) as final products. Chen et al. [47] developed a fast-setting calcium-MPC by introducing MPC

into CPC, which enhanced the anti-washout ability. The reaction may prohibit the liquid penetration to the cement, thereafter improving its washout properties, thus endowing the CPC/MPC with anti-washout ability.

3.4.5 Radiopacity

Cement leakage needs to be of concern when using injectable bone substitutes. Up to 70 distal radius cases on cement leakage have been reported. Thus, the radiological evaluation is necessary. Radiopacity refers to the relative resistance for electromagnetic radiation, such as X-rays, to pass through a material. The radiopacity assists in positioning of injectable implants in the body, detecting of injected implant over time, and forecasting possible failure. However, the intrinsic radiopacity of CPCs is limited, and typically the addition of a contrast agent is required for certain applications. Although ICPC approval for uses in vertebroplasty is considered intrinsically radiopaque, the radiopacity is sometimes not enough, and it can be troublesome to distinguish them from bone. Furthermore, both over-filling and under-filling of ICPC into the root canal often occur, causing serious problems during endodontic treatment. To fully fill the root canal, the obturation procedures are performed visually with close fluoroscopic monitoring. Radiography enables the filling of the canal to be monitored at later stages during root canal therapy and improves the clinical performance.

Radiopacifying agents are typically included in injectable biomaterial formulations to improve the radiopaque properties for radiography, computed tomography, and/or real-time fluoroscopy. Organic radiopaque agents can be ionic or nonionic compounds with covalently bonded iodine. Examples of organic compounds currently used as contrast agents include diatrizoic acid, metrizoic acid, iopamidol, and iohexol. Organic radiopaque agents are administrated orally for visualization of the organs, but their use is limited due to high cost and side effects.

Inorganic radiopaque agents (e.g., barium sulfate and zirconium dioxide) were also used in bone cements. However, they were not recommended due to the noted negative effects, such as causing bone resorption, decreasing the mechanical properties, and damaging biological functions [48]. Strontium ions have been reported to be used as radiopaque agents. Strontium fluoride and strontium chloride are mostly used for dental applications; however, they have a very low solubility and decrease the mechanical properties of the hardened end product significantly.

Accordingly, a resorbable radiopacifying agent in combination with high mechanical strength and biocompatibility is highly desirable for bone repair. Patent WO 2014/016707 A2 claimed that $SrBr_2$ and SrI_2 provided a better radiopaque effect than other strontium halides in injectable cement because of their higher molecular mass. This would result in the lower-amount uses of $SrBr_2$ and SrI_2 necessary to give the same radiopacity as other radiopaque has. Along with the higher intrinsic radiopaque and better water solubility, $SrBr_2$ and SrI_2 were biocompatible and favored the cell viability.

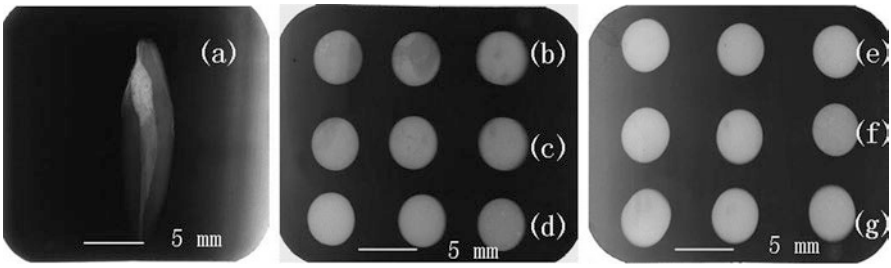

Fig. 3.7 Radiographic images of extracted human tooth, pure ICPC, and BD-ICPCs with different contents of bismuth salicylate basic: (**a**) extracted human tooth filling with pure ICPC, (**b**) pure ICPC, (**c**) BD-ICPC (5%), (**d**) BD-ICPC (10%), (**e**) BD-ICPC (15%), (**f**) BD-ICPC (20%), (**g**) BD-ICPC (25%) (Reproduced from Ref. [49] by permission. Copyright © 2010 Elsevier Ltd)

Bismuth salicylate basic was also introduced as an alternative radiopaque agent to ICPC in root canal fillings. The results showed that the radiopacity and sealability of ICPC were improved with the addition of bismuth salicylate basic due to its good radiopacity, stability, and insolubility (Fig. 3.7). It was noteworthy that this reagent also imparted the potent antimicrobial activity [49].

Radiopacity was always assessed by an X-ray device. After samples were exposed to X-ray for a period, the film was imaged in an automatic processor. The relative X-ray radiopacities of samples and the control group (e.g., extracted tooth) were judged visually. The image contrast between the sample region and the surrounding black region was used to evaluate the X-ray radiopacity of cement, which can be calculated according to Eq. 3.2 [50]. The image process software Adobe Photoshop® was adopted to test the grayscale of the sample region and the surrounding black region [51]. Ten pairs of regions, scattered as far as possible, were selected by random in both the sample and the black surrounding to measure their grayscales. Each region was averaged over an area of 5×5 pixels:

$$V = \frac{G_1 - G_2}{G_1} \tag{3.2}$$

As shown in Fig. 3.5, the radiographic image of pure ICPC was inhomogeneous and doped with some large drop shadows. The result indicated that the brightness of all radiographic images of BD-ICPC cements was increased, and the drop shadow areas were lessened, which indicated that the addition of BSB helped to improve the radiopacity of ICPC. In addition, contrasts were produced between the extracted human teeth and BD-ICPC. Measured contrasts of radiographs of pure ICPC and BD-ICPC were 0.55 ± 0.027 and 0.89 ± 0.008.

3.4.6 Suspension Stability

As stated earlier, nonaqueous ICPC facilitates the direct operation without the need of mixing on the spot and avoids the uneven performances caused by different operations. During this minimally invasive injection process, a well-dispersed suspension, with high solid loading of reasonably low viscosity to facilitate ICPC injected to the defect, is required. It was considered to be kinetically stable if the destabilization rate of the ICPC suspension is low enough during the expected lifespan. However, nonaqueous ICPC is a coexistent suspension system with solid and liquid phase. The interfacial energy between two phases is great; thus, the nonaqueous ICPC is an unstable system. In addition, CPC powders are easily aggregated in the nonaqueous solution due to the small particle size and large specific surface energy, which feed directly into inhomogeneity, flocculation, or precipitation of the ICPC suspension system. All these affect the injectability and the self-setting of ICPC and greatly limit the applications of ICPC in the clinic. Therefore, how to improve the long-term suspension stability of ICPC while maintaining the good slurry injection is an important issue to be addressed during the storage, transportation, and clinical application and the key to realize the large-scale production and applications in clinic.

Nonaqueous ICPC is a multicomponent, high viscosity, and opaque concentrated suspension, and it is sensitive to temperature and dilution. Two strategies are mainly conducted to study on the stable behavior. One is the measurement of the zeta potential of the particle surface to predict the stability of the dispersion [52]. This predictive method is effective for relatively simple preparations, but it cannot give information about the effects of the multiple components dissolved in the continuous phase on the flocculation behavior of particles. Aging tests are often performed. The products are evaluated for a long time under specific conditions (temperature, light, etc.). However, it is time consuming and cannot obtain objective and accurate data.

An instrument called Turbiscan LA-b^{Expert} (Formulation Co., Ltd., France) can directly measure the dynamic stability of suspension without dilution based on multiwavelength reflection. Compared with microscopy, particle size, and zeta potential analysis, the Turbiscan presents the advantage of being a nondestructive tool. It gives kinetic information on the process leading to phase separation and allows to detect two kinds of destabilization phenomena: particle migration which is often reversible by mechanical agitation and particle size variations (coalescence, flocculation). At the same time, the Turbiscan analyzes the unstable mechanism and speed at the beginning of the unstable phenomenon and measures the concentration change in the middle and bottom of the suspension with time [53].

The following are three behaviors (flocculation, coalescence, and subsidence) of thick suspension. The steady state of suspension indicates the particle size and solution concentration without change. Particle movement (emulsion oil rise or the solid particles settle) caused changes in the concentrations of the sample and the strength of the backscattering and transmission. Particle sizes change due to agglomeration

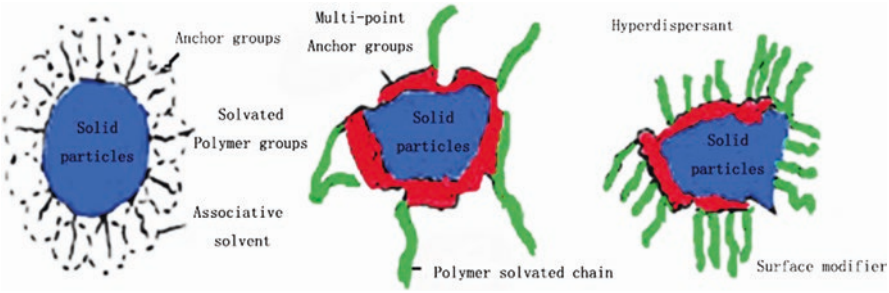

Fig. 3.8 Interactions of dispersant with particles in suspension system

of particles or droplets of flocculation, causing strength change of the backscattering and transmission. Therefore, the transmission and backscattering profiles contain overall (sedimentation and aggregation) kinetics information and the relative migration speed of the particles.

The suspension of ICPC is affected by agglomeration, dissolution, and thermal motion of the suspended particles. The rate of particle movement, thickness of precipitation phase, average particle size, the volume concentration of the disperse system, and a series of changes of the suspension system such as flocculation, emulsification, and sedimentation can be calculated according to changes in the transmission light of curve and the intensity of backscattered light. There are several ways to improve the suspension stability:

1. Constructing wettable surface of solid particles by the medium (Fig. 3.8). Solid particles are separated by the disperse medium to avoid agglomeration and sedimentation. Solid particles have a good dispersion effect in an aqueous medium, while they are poor in a nonaqueous phase. Therefore, new dispersants with anchoring groups and solvated chains for nonaqueous system should be explored.
2. Reducing the density difference between the dispersing medium and suspended substance. Sedimentation velocity is directly proportional to the density difference. Therefore, reducing sedimentation velocity is favored to enhance the suspension stability. A range of dispersants were added to modify stability. The result showed that polyacrylates, Darvan C, and Dispex A40 were the most effective dispersants for HA in water. Solsperse 3000 at low levels and Atsurf 3222 were the most effective among the 10 dispersants in alcohol. Solsperse 3000, a carboxylic acid-terminated polyester, was also the most effective dispersant in hexadecane [54].
3. Increasing viscosity and reducing relative molecular weight and particle diameter by grinding, homogeneity, or enzymatic hydrolysis is a common method to improve suspension stability. Sedimentation velocity is in proportion to the square of the radius of dispersed particles. To be more specific, the larger the particles are, the faster the sedimentation velocity is and vice versa.

Furthermore, the kinetic suspension stability can be attained by adding thixotropic agents [55–57]. Fumed silica formed the network structure when it was incor-

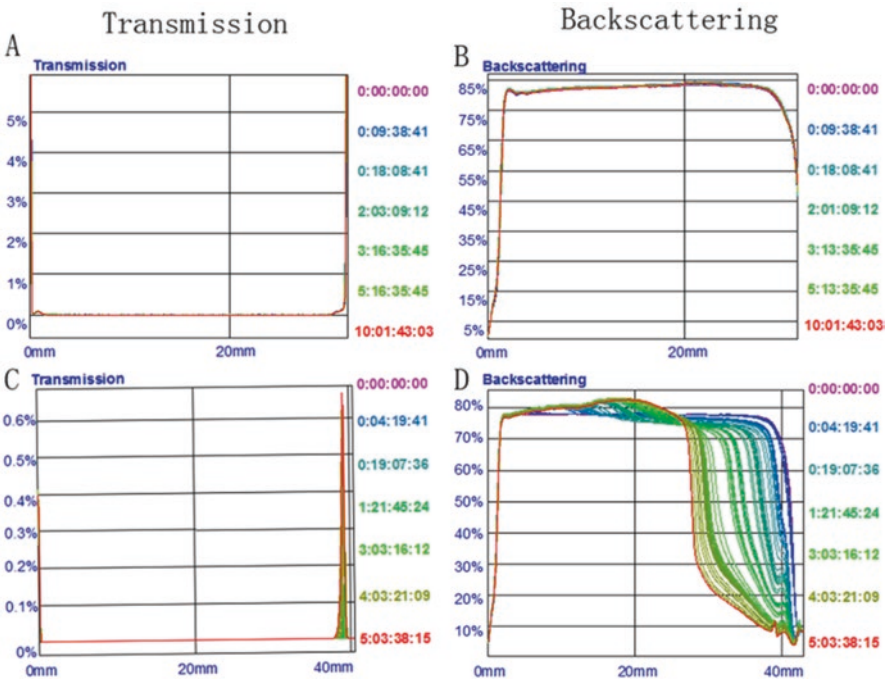

Fig. 3.9 (**a**) Transmission light curves of p-ICPC with sample height, (**b**) backscattered light curves of p-ICPC with sample height, (**c**) transmission light curves of n-ICPC with sample height, (**d**) backscattered light curves of n-ICPC with sample height (Reproduced from Ref. [58] by permission. Copyright © 2013 Springer Science + Business Media New York)

porated into n-ICPC pastes as a thixotropic agent. The suspension stability of p-ICPC was performed on the Turbiscan LA-b^{Expert}, and n-ICPC was used as a control. After a period of storage, tiny particles tend to settle down to the bottom of the sample cell due to the gravitation, which lead to the suspension destabilization. Interestingly, no sedimentation occurred in p-ICPC at room temperature (Fig. 3.9) even scanning for 10 days. Both T and BS in p-ICPC remained close to zero all through the sample cell, even at the top. On the contrary, the n-ICPC under the same condition stratified rapidly in two phases. The apparition of the nonaqueous phase was reflected by an increase in the T signals. Obviously, the suspension stability of p-ICPC was effectively improved. It is mainly due to the formation of the network structure [58].

3.4.7 Sealability

As root canal sealers, n-ICPC fills the narrow and irregular root canal. However, inadequate filling could lead to the fluid penetrant to the filling defects causing a periapical chronic inflammatory reaction and compromising the treatment success.

Therefore, the detection of leakage in vitro is widely used to evaluate the sealing efficiency with high clinical significance.

The leakage testing has a long history, and the first study on leakage was conducted in 1961 [59]. Based on the principles of leakage testing in other dental fields, leakage test were adopted and modified to test for implants leakage as well. Evaluation of the gap at the interface level with radiographs, SEM, or other optical means is a simple method to detect inadequacies in the connection. However, this testing is less reliable and could not reflect the real situation as the continuity and depth of the gap cannot be evaluated. Other accepted testing techniques are microbial leakage or bacterial seal testing [60], endotoxin or other molecular microleakage tests [61], spectrophotometric determination of dye penetration [62], electrochemical [63], glucose penetration model [64], and the gas permeability test [65]. The latter method not only provides information whether the implant leaks or not but also provides the leakage amount over time allowing for quantitative comparison among different implants systems.

Dye penetration was the most common method due to its simplicity [66] and intuitively observation of the linear penetration of the dye between the root canal filling and the walls. Methylene blue dye was used as a leakage marker because it is readily detectable under visible light, soluble in water, and easy to diffuse. Torabinejad et al. [67] stated that a material that prevents the small molecule's (dye) penetration should prevent larger substances like bacteria and their by-products. Camps and Pashley [68] reported that the dye penetration method saved much time. Ethylene blue microleakage was also used to estimate the sealability of ICPC in our study. The microleakage distance of methylene blue was measured directly with vernier calipers from the end of the root filling material to the end point of the dye in the canal. The result showed that the mean microleakage lengths of bismuth-doped ICPC were lower than that of pure ICPC, indicating that the former possessed better resistance against methylene blue dye penetration [69]. The good sealability was probably attributed to the improved injectability and formation of the gap-filling apatite deposits. On one hand, BSB enhanced the injectability of BD-ICPC and made BD-ICPC fill up any gaps induced during the material shrinkage phase and the dentinal tubules. On the other hand, the hydration product of ICPC wrapped a small amount of fine BSB, formed a closely packed structure, and made the BD-ICPC denser.

Rhodamine B was another dye to evaluate the sealability without suffering discoloration from filling materials, as occurs with methylene blue. Furthermore, the authors used vacuum to eliminate the air bubbles and make the dye easy to penetrate [70]. The result showed Rhodamine B was an effective method to evaluate the sealability of MTA and calcium hydroxide.

Bacteria are microorganisms that cause the infection of the root canal. To better simulate the oral environment, bacteria model in vitro is established closer to the clinical than the dye and the radioactive elements. Mortensen [71] proposed that microleakage can be measured by bacteria in place of dyes and radionuclides as a marker. Radionuclide penetration takes radionuclide-labeled particles as a penetration marker. Particles are protein, salt, urea, and other molecules. The labeled materials were introduced together with the solution for penetration and finally using the

self-development of the radioactive isotope. The depth of penetration usually displays in the X-ray. The biggest advantage of the liquid flow, microscope, and computer 3D reconstruction is to observe the microgap of the sample repeatedly and directly. Lyroudia [72] scanned the cross section of the root canal, studied the microseepage by the computer three-dimensional image reconstruction, and obtained the results from different visual angles.

3.5 Applications of ICPC in Hard Tissue Repair

3.5.1 Applications of ICPC in Bone Repairing

Due to the intrinsic biocompatibility, degradability of the material itself, and flexibility in shaping and injectability in practical use, CPCs serve as a promising candidate in bone tissue repairing and have already been proven as suitable substitutes in tissue engineering. Considering the specific chemical composition of CPCs, the combination of Ca and P boosts excellent osteoconductivity, which could stimulate further tissue regeneration, especially in hard tissue repairing areas such as spinal repair, root canal filler, or periodontal defect filling in physically damaged or pathological bone sites. In addition, the better understanding of setting behaviors of CPCs enables us to load drugs into the material in a tunable way to achieve a controlled release at the local site.

With the coming of aging society, the number of patients with vertebral compression fractures, vertebral metastasis, radius fracture, etc., triggered by osteoporosis is on the rise. The long-term pain or incapability to sit down has severely disturbed the normal life of people. Considering the low efficacy of medical treatment and unsuitability of internal screw fixation in patients with decreasing bone mechanical properties, percutaneous vertebroplasty by the use of ICPC to strengthen diseased vertebra could serve as a good therapeutic option.

A number of researchers have explored the superiority of ICPC in the field of bone repair. For example, Libicher [73] and coworkers applied resorbable CPCs in osteoporotic vertebral fractures and investigated the osseous integration. It was observed that the cured CPC materials demonstrated good integration with surrounding tissues and were gradually replaced by autologous bone tissue via osteonal ingrowth. Shigeo Ishiguro [74] applied ICPC in percutaneous vertebroplasty for osteoporotic compression fractures. Totally, 36 patients with osteoporotic fractures underwent percutaneous vertebroplasty using CPC. The clinical results showed that immediate pain relief was achieved, and the risk of vertebral body collapse and pseudarthrosis was reduced, demonstrating the effectiveness of ICPC.

Since 2003, our group [75] has carried out the minimally invasive therapy research of injectable and degradable calcium phosphate-based inorganic cements on vertebral fractures. It was found in the clinical use that ICPC boosted excellent

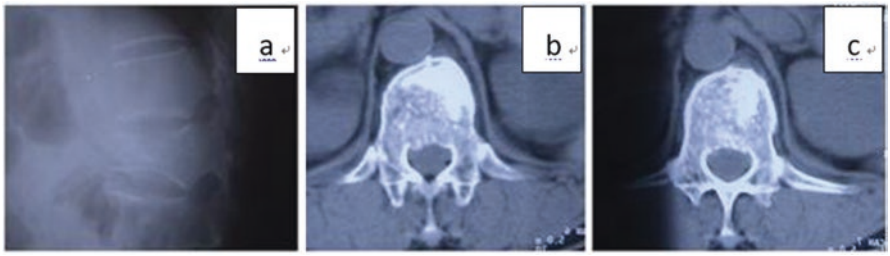

Fig. 3.10 Compression fracture treatment by injection of ICPCs (**a**) before surgery, (**b**) after injection, and (**c**) one month after surgery

injectability. The pastes could easily pass the syringe needle with 0.33 mm inner diameter, and the force needed to inject into centrum was less than that needed for PMMA. With synergetic modification of various compositions, the curing time of ICPC could be controlled under 18 min to achieve the initial strength and completely cured at body temperature (37 °C) after 24 h to achieve the highest strength, which could satisfy the requirements of surgeries. Meanwhile, the material possessed good water resistance, with the anti-washout rate of above 98%. The strength and tenacity were enhanced, with the increase of compressive strength from 22 MPa after 1 h to 47 MPa after 24 h, which was higher than the mechanical strength of similar imported products MIIG.

ICPC of TECP/DCPA system prepared by our group has been used in clinical trials. The mechanical strength of ICPC is 20 MPa higher than the average commercial products, ranging from natural spongy bone and cortical bone, self-set within 3–15 min, and achieves 85% of the highest compression strength after 4 h. Meanwhile, the ICPC system exhibits an outstanding injectability with excellent bonding to bone tissues after setting.

In one case, ICPC was employed in percutaneous vertebroplasty. As it was inferred from the CT image (Fig. 3.10), ICPC materials were well filled into the damaged cavity and demonstrated good adhesion to surrounding bone tissues, greatly promoting the therapeutic effect.

ICPC was also injected into the vertebral bone trabecular gap of fresh bodies to further investigate biomechanics. Only 8–10 psi was needed during the injection, which was greatly lower than that of PMMA (20–40 psi), proving again its excellent injectability. The CPCs could self-set within 8–15 min at the gap. After injection, the loading capacity has increased to 8000 N, 52.4% higher than the original centrum, showing that the mechanical property could satisfy the clinical need.

To sum up, the ICPC could be considered as an outstanding material, especially in spinal repairing such as percutaneous vertebroplasty. Of course, to meet with clinical requirements, injectable bone cements whose viscosity versus time from commencement of mixing characteristics are consistent with the aforementioned requirements of various patients still have to be developed [74]. In addition, more clinical trials are needed to further identify the effectiveness of ICPC materials.

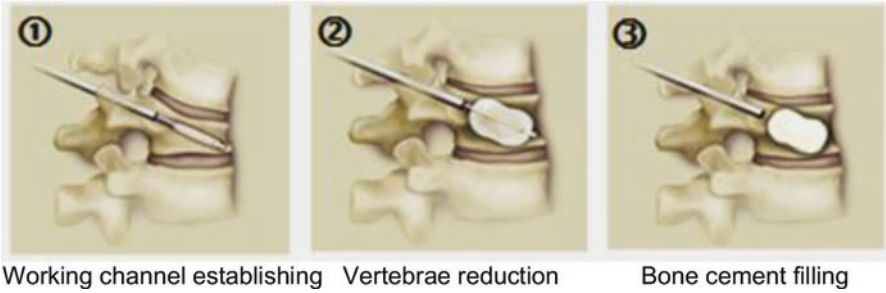

Fig. 3.11 Operating process of percutaneous kyphoplasty

3.5.2 Percutaneous Vertebral Device for Minimally Invasive Treatment of ICPC

In minimally invasive vertebral body forming technology, designing reasonable supporting equipment and establishing an effective working channel are the keys to ensure the point injection of bone cement and restore the vertebral body height. In recent years, percutaneous kyphoplasty (PKP) rapidly developed, that is, the percutaneous and pedicle firstly imbedded the inflatable balloon into the vertebral body, expanding the balloon and squeezing the damaged vertebral body to reduce the fracture vertebrae with a safe and effective space, followed by venting out of the balloon and filling the bone cement at a low pressure (Fig. 3.11). Using the technology of PKP can restore the vertebral height, enhance the vertebral strength, and reduce postoperative pain. More importantly, filling CPC into the vertebrae under low pressure can effectively lessen the leakage of cement to reduce complications. However, obtaining a miniature, thin-walled balloon and ensuring the sealing connection between balloon and catheter is very difficult. The existing cardiovascular dilatation balloon cannot withstand the high pressure above 350 psi which is considered to be the minimum pressure of vertebral minimally invasive surgery. Additionally, the deformation of balloon should be limited to keep stable dimensions under the desired high pressure. And the cardiovascular dilatation balloon cannot meet this requirement.

In order to meet the special requirements of inflatable balloon in the process of vertebral minimally invasive treatment, our group invented the process technology of multistage ring-shaped rotary expansion and radial double-sided tensile to prepare high-resistive tear balloon [76]. On account of thermoplastic property of polyurethane (TPU) elastomer, our group produced the isotropic TPU materials through multiple bidirectional expansion orientation techniques. The rotational expansion and radial stretching of TPU lead to the orderly alignment of polymer chains in a circumferential and radial direction, respectively. This process enhances and stabilizes the tear strength and dimensional stability of the thin-walled balloon. The balloon can still withstand the pressure environment more than 350 psi when the wall thickness is about 0.2 μm, and the balloon possesses excellent mechanical strength

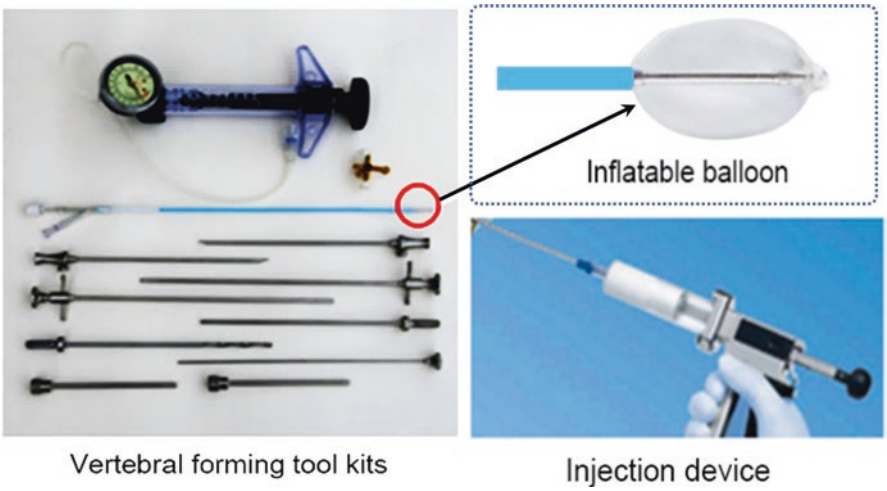

Fig. 3.12 The devices and tools for percutaneous kyphoplasty

which can withstand the sharp contact pressure like bone fragments without damage. Moreover, the balloon can rebound back to the size before expansion for facilitating removal. On the other hand, the high-frequency (>100 kHz) welding technology was utilized to achieve the seal connection between the balloon and the catheter (Fig. 3.12). The finally prepared balloon catheter has a maximum tolerated pressure of no less than 400 psi, which can meet the requirements of PKP operation.

3.5.3 Operational Techniques for Centrum Minimal Invasive Treatment in Clinical Use

Posterior pedicle screw is one of the common methods employed in the treatment of thoracic and lumbar fractures. However, due to the osteoporosis of elderly patients, the implant could suffer from the poor stability, enlarged porosity after resetting of fracture, and collapse of the centrum after recovery. All the above defeats could induce the rapid increase of stress on the posterior pedicle screw, thus leading to the high failure of the fixation due to the loosening and rupture of screw. Meanwhile, the screw expands the fixation sites, and only the cortical bones surrounding the centrum are restored. By combining the centrum minimal invasive treatment with pedicle screw fixation, a new clinical operational technique has been proposed and reduced stress of pedicle screw when working alone, lowered the failure rate of inner fixation, and maintained better restoration of fractured part. Compared with PMMA, ICPC has less influence on the near intervertebral disc after centrum strengthening, thus significantly improving the load distribution of spinal stress field and displacement field.

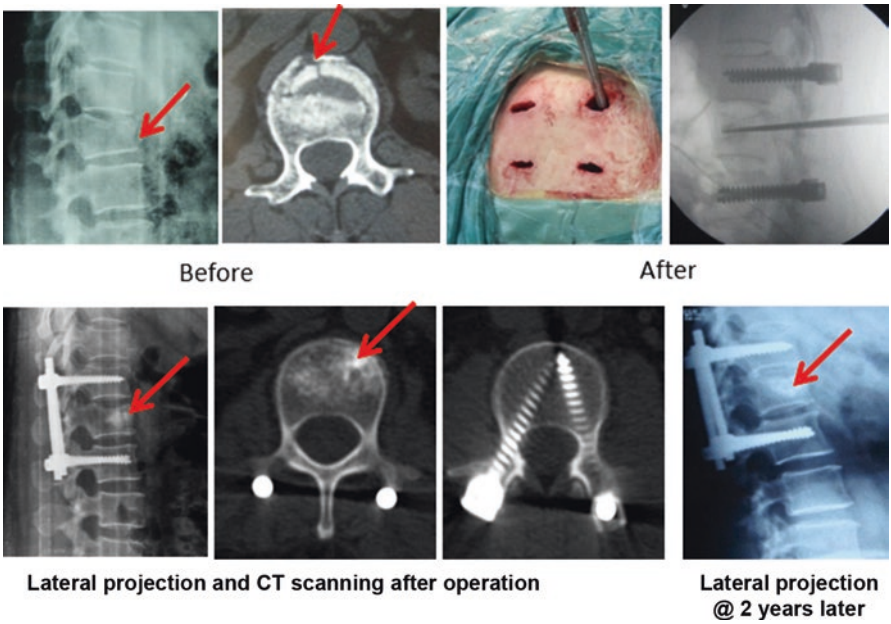

Fig. 3.13 45-year-old female, vertebral pedicle screw fixation, and PVP (ICPC from Rebone, Shanghai, China) (Reproduced from Ref. [77] by permission. Copyright © 2017)

Patients with fresh single-segment thoracolumbar burst fracture and without neurological symptoms are selected for pedicle screw fixation and percutaneous vertebroplasty. The clinical results reveal that the combination of centrum minimal invasive treatment with pedicle screw fixation cannot only improve the restoration of fractured centrum but also maintain the height after operation to prevent any losses that lead to fixation failure. Compared with traditional methods, the new approach reduces the operation time and accelerates the recovery, thus opening up a new door for the safe and effective treatment of osteoporotic vertebral fractures and thoracolumbar vertebral burst fractures. In one typical case (Fig. 3.13), a 45-year-old female with first lumbar vertebral burst fracture has taken the combined treatment of vertebral pedicle screw fixation and percutaneous vertebroplasty (ICPC produced by Rebone, Shanghai). Pain score dropped to 2 points immediately and fell to 0 after treatment for 3 months. ICPC had a lower occurrence rate of pulmonary fat embolism than PMMA. The central and anterior vertebral body height significantly increased, and the strength of catagmatic vertebra restored to two times, while rigidity increased 15–20%. It kept shapes and internal structure of the vertebra, preventing micromotion in the vertebra and providing a stable internal environment for recovery. The results confirmed that percutaneous vertebroplasty with ICPC is a good choice for the treatment of acute thoracolumbar osteoporotic vertebral compression fractures.

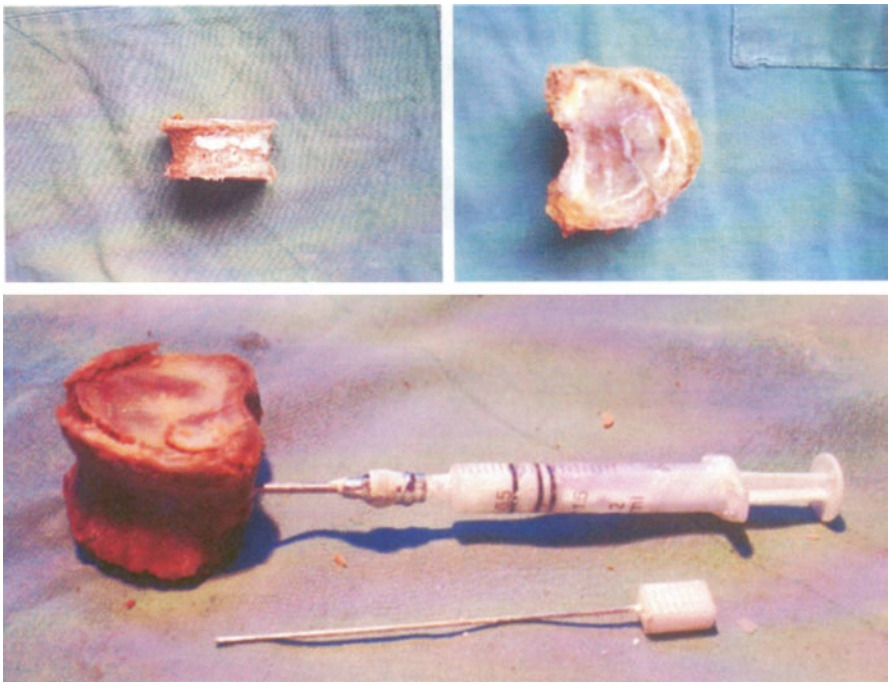

Fig. 3.14 Injection of ICPC into centrum (Which is reproduced from Ref. [78] by permission)

Biomechanics in percutaneous vertebroplasty after fracture was also investigated. During the trails on fresh bodies, ICPC was injected into gaps resulted from fracture, and cavity formed after the removal of the centrum (Fig. 3.14). After the injection of ICPC and thereafter hardening process, the load-bearing capacity of the repaired site was 2275 N, a 16.7% increase compared to 1950 N of the original one. Meanwhile, the stiffness also increased by 11.1% after the injection of ICPC materials. As we can see from the CT images in Fig. 3.15, the materials presented good adhesion to the surrounding bone tissues.

3.5.4 Application of ICPC as Root Canal Fillers

Root canal filler serves as a good therapeutic method for dental-related diseases, including periapical or pulposus problems. A careful choice of filler material and a good performance in filling process remain the most important factors in clinical applications. ICPC, with excellent biocompatibility and osteoconductivity, has also been extensively employed in dental field.

An ideal root canal filler is required to be nontoxic, no stimulation, and no obvious postoperative inflammatory reaction when it contacts with periapical tissue. It

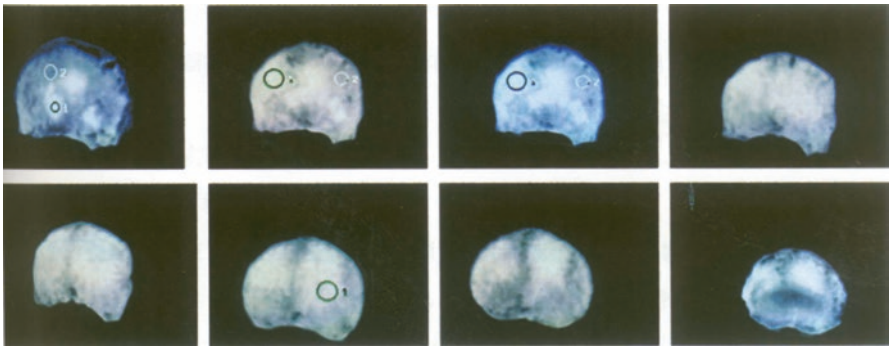

Fig. 3.15 CT images of ICPC filling areas (Which is reproduced from Ref. [78] by permission)

should offer adherence to dentinal walls of the retrograde preparation, periradicular tissue tolerance, and bioactive promotion of healing. Meanwhile, the filling material should not corrode or be electrochemically active. It should also be kept in mind that easiness to manipulate, dimensional stability, non-absorbability, as well as no penetration by bacteria must be considered during the selection of materials [79].

A DCPD-CaO-based hydraulic CPC was investigated for its antimicrobial activity and tightness in root canal filling [80]. In the following studies, it was found that in comparison to traditional calcium hydroxide pastes, the CPC material demonstrated better mechanical properties and provided a fluid-tight sealing [81]. Sealing property is considered as one of the most important parameter in root canal filler and to some degree determines the final therapeutic effect [82]. As a result, the performance in sealing ability of different fillers was also evaluated in the report. It was shown that CPC paste containing dicalcium phosphate anhydrous and dicalcium phosphate dihydrate exhibited good sealing ability against dye penetration, suggesting the capability to provide adequate seal of the canal. BD-ICPC exhibited improved plasticity, self-setting, radiopacity, sealability, and potent antimicrobial activity. In addition, BD-ICPCs afforded a uniform and tight adaptation to the root canal wall. It is expected to be used as a novel root canal filling material.

The tissue response of CPC was studied [83]. The results illustrated that, in the histological study in dogs, varying tissue reactions in contact with CPCs after implantation did not lead to severe inflammation, confirming the biocompatibility and nontoxicity of the materials. Hong CY [84] injected ICPC into monkeys' incisor and overbrimmed it to periapical tissues. Only mild stimulation reaction was observed after 1 month of treatment and no adverse reaction after 5 months. Goodel [85] made CPC into sealer of root canals and filled the gutta-percha point with Grossman's cement by high pressure. The control group only sealed gutta-percha points with Grossman's cement in pressure. The results of linear dye penetrant test in two groups showed that the experimental group had less dye infiltration than the control group in the root canal. Then the team used CPC to carry on leak proofness tests with zinc oxide eugenol sealer (ZOE) and gutta-percha points sealed with

Grossman's cement, respectively, proving that CPC as root filling material has good properties of sealing [86, 87]. Tchaou [88] compared six kinds of dental materials, such as CPC, formaldehyde cresol (FC), calcium hydroxide, zinc oxide, etc., for antibacterial effect against 21 kinds of bacteria in the root canal. The results indicated that the material containing CPC and FC has the strongest antibacterial ability, especially for anaerobic gram-negative bacteria.

Though ICPC has a series of comprehensive advantages and has already been applied in practical use, there is still a long way to go to further understand the in vivo behaviors and further confirm the therapeutic effect.

3.5.5 Periodontal Defect Filling

The periodontium is a complex anatomical structure composed of both hard (bone and cementum) and soft (periodontal ligament) connective tissues [89]. A periodontal wound is always created along with a series of periodontal lesions which have shown to be a high risk of disease progression in subjects who had not received systematic therapy [90], while deep intraosseous defects represent a major challenge for the clinician.

CPCs are identified as an emerging bone substitute materials for osseous augmentation due to its unique combination of osteoconductivity, biocompatibility, and mouldability. Moreover, the injectable CPC is important for minimally invasive surgery and optimal defect filling in clinical applications of periodontal defect to provide intimate adaptation to the bone lesions and cracks.

Nowadays, the technologies of periodontal defect repair have become increasingly mature, and a series of preclinical or clinical evaluation criteria and models were proposed such as socket healing, supra-alveolar periodontal defects, and furcation and fenestration defects [91]. ICPC was treated for pulpal and periapical diseases. The researchers suggested that the materials could close root canal and prevent bacteria invasion without adverse reactions. There was no side effects including tooth discoloration, volume change, postoperative pain, local gums bleeding, edema, and so on [92]. Yoshinori Shirakata et al. [93] concluded that the filling volume and stiffness of CPC may compromise the clinical outcomes for periodontal intrabony defects. However, the study failed to demonstrate any superior clinical outcomes for the CPC group compared to the open flap debridement group, whereas radiographs revealed more favorable results in the CPC group. In addition, the purpose of periodontal regeneration procedures is not just to obtain a bone filling of the defect, but a regeneration of all the periodontium. To improve the injectability of materials and their stability in the osseous defects, researches developed a composite based on silanized hydroxypropyl methyl cellulose (Si-HPMC) and biphasic calcium phosphate (HA and β-TCP), which obtained an exciting repair results in dog's critical size furcation defects [93].

Although injectable CPC has been used in clinical for a long time, however, drawbacks still existed such as easy to collapse and low osteogenesis. To address these issues, CPC-based composites were developed: some were studied in vivo experiments, and some were assessed by preclinical evaluations.

3.6 Summary and Perspectives

Injectable calcium phosphate cements have received much attention in recent years due to their numerous potential advantages such as in situ self-setting, precision injection, no exothermic heat release during the hydration process, minimal damages to tissues, and great relief of patients' suffering.

However, there are still many issues that restrict their wider clinical applications. For instance, the requirements regarding viscosity are conflicting. Ideally, the viscosity of ICPCs should be low and easy for injection through the cannula but high enough after implantation in bone defect to prevent from the tissue fluid to washout. Future research should focus on the improvement of the strength and in the meantime the injectability. A injectable cement with good injectability possesses the higher ratio of liquid to solid, thereafter having lower strength after setting. Potential solutions to overcome the shortcoming are to introduce some setting accelerators. Developing an injectable biomaterial for the encapsulation of viable cells and mimicking the 3D structure of natural bones are the potentially fruitful direction of injectable cements for hard tissue repair. Furthermore, a comprehensive internationally accepted standard should be developed to characterize the injectable bone cements. This standard should document procedures for determining not only the properties of ICPCs but also the guidance on how to use them in clinical applications.

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