DESIGN OF CERAMIC-BASED CEMENTS AND PUTTIES FOR BONE GRAFT SUBSTITUTION

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Abstract

In the last 15 years, a large number of commercial ceramic-based cements and putties have been introduced as bone graft substitutes. As a result, large efforts have been made to improve our understanding of the specific properties of these materials, such as injectability, cohesion, setting time (for cements), and *in vivo* properties. The aim of this manuscript is to summarize our present knowledge in the field. Instead of just looking at scientific aspects, industrial needs are also considered, including mixing and delivery, sterilization, and shelf-life.

Keywords: Putty, cement, bone graft substitute, calcium phosphate, injectable.

Note: This article is based on a chapter published in Injectable biomaterials, edited by B. Vernon, published by Woodhead Publishing Limited in 2010 (the original chapter is copyright Woodhead Publishing Limited 2010)

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Introduction

A few millions patients per year need a bone graft or bone graft substitute to repair a bone defect resulting from an injury or a disease. A large number of bone graft substitutes can be used: unprocessed or processed allogenic bone, animal-derived bone substitutes and synthetic bone substitutes, mostly ceramics (Bauer and Muschler, 2000).

Even though the first studies dealing with ceramic bone substitutes are more than 100 years old (Albee and Morrison, 1920; Dreesmann, 1892), it is only in the 1970s that research soared (Cameron *et al.*, 1977; Hench, 1980; Hulbert *et al.*, 1970; Jarcho *et al.*, 1976; Klawitter and Hulbert, 1971; Nery *et al.*, 1978; Roy and Linnehan, 1974; White *et al.*, 1972). In the early days, studies were mainly focused on porous blocks and granules (Cameron *et al.*, 1977; Hulbert *et al.*, 1970; Klawitter and Hulbert, 1971; Nery *et al.*, 1978; Roy and Linnehan, 1974; White *et al.*, 1972)). However, the discovery of calcium phosphate cements (CPC) in 1982-1983 (Brown and Chow, 1983; LeGeros *et al.*, 1982) opened up a new era in which the handling properties of bone graft substitute became of paramount importance.

Several new approaches have been proposed to improve them. For example, Hanker (Hanker *et al.*, 1986) combined in 1986 Plaster of Paris with calcium phosphate granules to obtain an injectable and setting biphasic paste. In 1987, Klein *et al.* proposed to mix a sodium alginate solution with β-tricalcium phosphate (β-TCP; $Ca_3(PO_4)_2$; see Table 1) granules (0.5-1.0mm in diameter) to obtain an injectable and hardening paste (hardening of the alginate molecules through crosslinking with Ca ions) (Klein *et al.*, 1987). Similarly, Gerhart *et al.* (Gerhart *et al.*, 1988; Gerhart *et al.*, 1989) presented in 1988 a system consisting of gelatine solution, β-TCP granules (0.355-0.60mm) and a crosslinker. In the mid 1990s two commercial CPC formulations were introduced (Constantz *et al.*, 1995; Kveton *et al.*, 1995a; Kveton *et al.*, 1995b). These were followed by more than a dozen other commercial CPC formulations (Table 2). Recently, efforts towards composites of hydrogels and bone substitutes (Chan *et al.*, 2002; Chazono *et al.*, 2004; Dupraz *et al.*, 1998; Grimandi *et al.*, 1998; Ito, 1991; Maruyama *et al.*, 1995; Momota *et al.*, 2002; Pompili *et al.*, 1998) have been intensified and several products have been launched (Table 3). These efforts are expressed by a rapid increase of the number of publications. For example, a search in "Scopus" (www.scopus.com) using the two keywords "Injectable" and "Ceramic" shows that almost 350 publications were published in 2009 (Fig 1). Combining "Putty" with "Ceramic" leads to a lower number of publications but the evolution is remarkably similar.

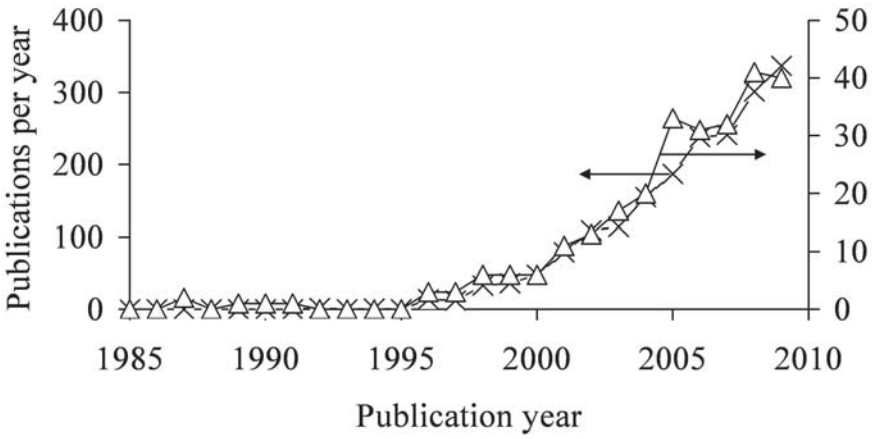

Figure 1: Number of articles cited per year in scopus (www.scopus.com) when selecting the following keywords (search in all fields): (x) "Injectable" and "Ceramic"; (Δ) "Putty" and "Ceramic". State on May 31, 2010.

The aim of this manuscript is to summarize our present knowledge in the field. All types of pasty bone substitutes involving ceramics are considered here. The spectrum goes from non-setting hydrogel-granule putties to CPCs. The term "ceramic" refers generally to non-metallic inorganic materials obtained at high temperature. Here, a broader definition is used since cements are consolidated at or close to room temperature. Therefore, "ceramic" here refers simply to non-metallic inorganic synthetic materials. As a result, all bone-derived pastes are excluded from this manuscript. Importantly, instead of just looking at scientific aspects, such as physico-chemical and biological properties, industrial needs are also considered, including mixing and delivery, sterilization, and shelf-life.

Rheological Properties

The rheological properties of a bone substitute paste are obviously very important. These include the injectability, the cohesion and the viscosity. Regarding injectability, our understanding has improved markedly in recent years (Bohner and Baroud, 2005; Habib *et al.*, 2008). When a paste which is a biphasic mixture of a finely divided ceramic (powder, granules) and a liquid is submitted to a pressure gradient, the liquid may flow faster than the solid, resulting in local changes of the paste composition. Specifically, the paste present in the region of the highest pressure (e.g., close to the plunger of a syringe) may become so depleted in liquid that the biphasic mixture in this zone is not longer a paste, but a wet powder (Bohner and Baroud, 2005; Habib *et al*., 2008). Contrarily, the paste in the zone of the lowest pressure (e.g. at the cannula tip) is enriched in liquid. Since these effects are dynamic, the size of the zone depleted in liquid (wet powder) increases during injection, eventually reaching the tip of the injection device and plugging it. This phenomenon is generally referred as filter-pressing, phase separation, or phase migration.

Fortunately, filter-pressing can be reduced or even eliminated by decreasing the particle size of the finely divided solid (powder, granules) (Bohner and Baroud, 2005), using rounder particles (Ishikawa, 2003), using additives to increase the viscosity of the mixing liquid (Andrianjatovo *et al.*, 1995; Bohner and Baroud, 2005), or manipulating the plastic limit and liquid-to-solid ratio (LSR) of the paste (Bohner and Baroud, 2005). Concerning the latter strategy, it has been demonstrated that the injectability increases when the difference between the paste LSR and the plastic limit (minimum amount of liquid to add to a solid to obtain a paste) increases (Bohner and Baroud, 2005). This can be either achieved with an increase of the LSR (Bohner and Baroud, 2005; Burguera *et al.*, 2008), or with a decrease of the plastic limit, for example by adding citrate ions or polyacrylic acid into the mixing liquid (Barralet *et al.*, 2004; Bohner and Baroud, 2005), or by optimizing the particle size distribution of the solid (Gbureck *et al.*, 2005b).

Importantly, there is presently no agreement in the scientific community about the meaning of injectability. For many authors, injectability is a concept related to the force that has to be applied to a syringe in order to inject the paste, independently of the fact that the force is a function of syringe size (Khairoun *et al.*, 1998). A paste is declared non-injectable if the paste cannot be injected with an arbitrary force (generally 100 N) using an arbitrary syringe geometry. Another approach is to define the cannula diameter below which the paste cannot be fully injected anymore (Nilsson *et al.*, 2008). This definition is very useful for specific applications such as minimally-invasive surgery and robocasting, for which the paste has to be injected through very thin cannulae (typically < 0.1mm). In the present document, the injectability is related to the ability of a paste to remain homogeneous under pressure, since phase separation is the cause of filter-pressing. So, according to this definition, injectability is still related to a given geometry, but not anymore to a force. In other words, an injectable paste according to the definition used here might be found non-injectable according to the definition of Khairoun *et al*. (Khairoun *et al*., 1998).

The second rheological property that should be carefully considered while designing a ceramic bone substitute is the paste cohesion (= cohesiveness, "non-

Table 1. Main calcium phosphate compounds. The first 6 compounds precipitate at room temperature in aqueous systems. The last 6 compounds are obtained by thermal decomposition or thermal synthesis. The 6 columns contain the name, the corresponding chemical formula, the Ca to P molar ratio, the mineral name, and the typical acronym, respectively. When x > 0 in the chemical composition of "precipitated hydroxyapatite", one talks also about "calcium-deficient hydroxyapatite" (CDHA). Generally, x = 1 so that CDHA has in most cases the composition $Ca_9(HPO_4)(PO_4)_5OH$.

Name	Formula	Ca/P	Mineral	Symbol
Monocalcium phosphate monohydrate	$Ca(H_2PO_4)_2·H_2O$	0.50	-	MCPM
Dicalcium phosphate	$CaHPO_4$	1.00	Monetite	DCP
Dicalcium phosphate dihydrate	$CaHPO_4·2H_2O$	1.00	Brushite	DCPD
Octocalcium phosphate	$Ca_8H_2(PO_4)_6·5H_2O$	1.33	-	OCP
Precipitated hydroxyapatite[1]	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$	1.33-1.67	-	PHA
Precipitated amorphous calcium phosphate	$Ca_3(PO_4)_2·nH_2O$ where n = 3-4.5; 15-20% H_2O	1.50	-	ACP
Monocalcium phosphate	$Ca(H_2PO_4)_2$	0.50	-	MCP
α-Tricalcium phosphate	$α-Ca_3(PO_4)_2$	1.50	-	α-TCP
β-Tricalcium phosphate	$β-Ca_3(PO_4)_2$	1.50	-	β-TCP
Sintered hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	1.67	Hydroxyapatite	SHA
Oxyapatite	$Ca_{10}(PO_4)_6O$	1.67	-	OXA
Tetracalcium phosphate	$Ca_4(PO_4)_2O$	2.00	Hilgenstockite	TetCP

[1](x may vary between 0 and 2)

decay"). Specifically, it is the ability of the paste to keep its geometrical integrity in an aqueous solution. For a cement, a bad cohesion may prevent setting and may lead to negative *in vivo* reactions due to the release of microparticles (Miyamoto *et al.*, 1999). Since a high cohesion is the result of strong attractive forces between particles, factors enhancing van der Waals forces (attractive) and decreasing electrostatic forces (repulsive) can be used to improve cohesion. These include a decrease of mean particle size and LSR, and an increase of ionic strength of the mixing solution, (Bohner *et al.*, 2006a). Another approach is to increase the viscosity of the mixing liquid using hydrogels (Andrianjatovo *et al.*, 1995; Bohner *et al.*, 2006a; Cherng *et al.*, 1997). Similarly to cement pastes, it is likely that non-setting pastes consisting of nano- or microparticles (what could be called "mineral suspension") may produce negative biological reactions due to particle release. For pastes consisting of milliparticles ("granules"), a loss of cohesion during implantation may require an intensive washing to remove all loose particles.

So far, relatively little is known about ways to control the viscosity of cement pastes. In fact, to talk about viscosity is an approximation of reality: calcium phosphate pastes are generally non-Newtonian fluids and as a result, the viscosity is a function of shear forces (Baroud *et al.*, 2005). Furthermore, cements have transient properties meaning that the viscosity of a cement paste is a function of shear and time (Liu *et al.*, 2006). Generally, calcium phosphate pastes are thixotropic (shear-thinning) (Baroud *et al.*, 2005; Liu *et al.*, 2006). Both an increase of LSR and an increase of particle size decrease the paste viscosity (Baroud *et al.*, 2005; Liu *et al.*, 2006). Additives are also known to affect viscosity. For example, citrate ions or poly(acrylic acid) decrease the particles interaction and hence decrease viscosity and cohesion (Baroud *et al.*, 2005).

Handling and Delivery

The handling of a product is of paramount importance for its commercial success. In the case of injectable ceramics, the following aspects have to be carefully looked at: mixing, transfer into a delivery system, and delivery. Besides, the product should be versatile and visible radiologically. These various aspects are discussed hereafter.

There are three categories of products regarding mixing: pre-mixed (= ready-to-use) products (Table 3), products that are mixed during delivery (e.g., "VitalOs" in Table 2), and products that have to be mixed prior to use (Tables 2 and 3). Even though pre-mixed products appear very attractive, each of the latter three categories has specific advantages and disadvantages. So, it is important to understand them during the design process of the product. Here is a quick review.

Pre-mixed products are the easiest to use because they do not require any mixing and any transfer into an appropriate delivery system. Moreover, there is no time constraint to use the product once it is open. However, pre-mixing is not a versatile approach to deliver a product since the mixture composition is already pre-defined. Moreover, it is not adapted to CPCs formulations. Presently, only two methods have been proposed to package ready-to-use cement formulations. First, the reactive cement components are combined with a non-aqueous liquid to form a non-reactive pasty mixture (Aberg *et al.*, 2010; Carey *et al.*, 2005). Reaction occurs then *in vivo*, when the non-aqueous liquid is slowly replaced with physiological fluids. Unfortunately, the setting reaction is difficult to control and the mechanical properties are poor. The second approach is to freeze down the cement components (Grover *et al.*, 2008). However, it is not clear how the storage and handling could be controlled (freezing at -80ºC). Another interesting approach consists of mixing two reactive liquids during their injection by means of a

Table 2. List of commercial ceramic cements with the producer, product name, composition (when available) and main end-product. The main end-product of the reaction can be either an apatite (calcium-deficient, carbonated, etc…), brushite (= DCPD) or gypsum ($CaSO_4 \cdot 2H_2O$; CSD). Denominations: see Table 1 for details.

Producer	Product name	Composition	Product
AG Digital Technology Corp	A-GRIX	Powder: calcium sulphate hemihydrate powder ($CaSO_4 \cdot ½H_2O$; CSH) & calcium sulphate granules; Solution: Aqueous solution[1]	Gypsum
Berkeley Advanced Biomaterials (US)	Cem-Ostetic™	Powder: calcium phosphates (details unknown); Solution: Sterile water[2]	Apatite
	Tri-Ostetic™	Powder: calcium phosphates (details unknown); Solution: Sterile water[2]	Apatite
Biocomposites Ltd (GB)	Genex®	Composition: could not be found[1]	Gypsum
Biomatlante (FR)	MCPC	Powder: mainly α-TCP, ACP, BCP = biphasic calcium phosphate (composite between HA and β-TCP); Solution: phosphate buffered solution (Khairoun *et al.*, 2005)	Apatite
Biomet (US) Interpore (US) Walter Lorenz Surgical (GER)	Calcibon®	Powder: α-TCP (61%), DCP (26%), $CaCO_3$ (10%), PHA (3%); Solution: H_2O, Na_2HPO_4 (Khairoun *et al.*, 1999)	Apatite
	Mimix™	Powder: TetCP, α-TCP, trisodium citrate ($C_6H_5O_7Na_3 \cdot 2H_2O$); Solution: H_2O, citric acid ($C_6H_8O_7$)[2]	Apatite
	Quick Set Mimix™	Powder: Calcium phosphate powders, $Na_3C_6H_5O_7 \cdot 2H_2O$; Solution: Citric acid aqueous solution[2]	Apatite
	Bone Plast® QS	Powder: CSH ($CaSO_4 \cdot ½H_2O$); Solution: sterile aqueous solution[2]	Gypsum
BoneSupport AB (SWE)	Cerament™	Powder: $CaSO_4 \cdot ½H_2O$ (60%), HA (40%); Solution: Aqueous solution of an iodine radiopacifier (http://www.bonesupport.com/)	Gypsum
Calcitec (US)	Osteofix	Powder: calcium phosphate and calcium oxide powders; Solution: phosphate buffer[2]	Apatite
ETEX (US)	? -BSM; Embarc; Biobon	Powder: ACP (50%), DCPD (50%); Solution: Unbuffered aqueous saline solution (Lee *et al.*, 1999; Tofighi *et al.*, 2001)	Apatite
	β-BSM	Composition: could not be found[1] (it has apparently a higher compressive strength and better injectability than α-BSM)	Apatite
	γ-BSM	Composition: could not be found[1] ("putty" consistency)	Apatite
	OssiPro	Composition: could not be found[1]; The cement is claimed to be macroporous after hardening[2]	Apatite
	CarriGen	Composition: synthetic calcium phosphate, sodium carboxymethyl cellulose, sodium bicarbonate, and sodium carbonate[2]	Apatite
Futura Biomedical (US)	OsteoCure	Powder: $CaSO_4 \cdot ½H_2O$; Solution: sterile mixing solution[2]	Gypsum
Graftys (FR)	Graftys® HBS	Powder: mainly ? -TCP, ACP, BCP = biphasic calcium phosphate (composite between HA and β-TCP); Solution: phosphate buffered solution (Khairoun et al., 2005)	Apatite
	Graftys® Quickset	Composition: calcium phosphate salts, hydroxypropylmethylcellulose (HPMC), and phosphate-based aqueous solution[2]	Apatite
Kasios (FR)	Jectos Eurobone®	Powder: β-TCP (98%), $Na_4P_2O_7$ (2%); Solution: H_2O, H_3PO_4 (3.0M), H_2SO_4 (0.1M) (Frayssinet *et al.*, 2000)	Brushite
	Jectos+	Composition: could not be found (likely to be close to that of Jectos)[1] (http://www.kasios.com/doc-pdf/JECTOS%2B699ed03-frgb.pdf)	Brushite
Kyphon (US)	KyphOs™	Powder: ? -TCP (77%), $Mg_3(PO_4)_2$ (14%), $MgHPO_4$ (4.8%), $SrCO_3$ (3.6%) ; Solution: H_2O, $(NH_4)_2HPO_4$ (3.5M) (Mulliez and Wenz, 2002)	Apatite
Lifecore (US)	CalMatrix	Powder: 90% $CaSO_4 \cdot ½H_2O$ and 10% carboxymethylcellulose; Solution: could not be found[2]	Gypsum
Mitsubishi Materials (J)	Biopex®	Powder: α-TCP (75%), TetCP (20-18%), DCPD (5%), HA (0-2%) Solution: H_2O, Sodium succinate (12-13%), sodium chondroitin sulfate (5-5.4%) (when two values are indicated, the first value stems from reference (Kurashina *et al.*, 1997) and the second value from reference (Tanaka *et al.*, 2003))	Apatite
	Biopex®-R	Powder: α-TCP, TetCP, DCPD, HA, $Mg_3(PO_4)_2$, $NaHSO_3$ Solution: H_2O, Sodium succinate, sodium chondroitin sulfate (Tanaka et al., 2003)	Apatite
Orthogen Corporation	DentoGen	CSH powder and aqueous solution	Gypsum
Produits Dentaires SA (CH) CalciphOs (CH)	VitalOs[4]	Solution 1: β-TCP (1.34g), $Na_2H_2P_2O_7$ (0.025g), H_2O, salts (0.05M pH 7.4 PBS solution); Solution 2: MCPM (0.78g), $CaSO_4 \cdot 2H_2O$ (0.39g), H_2O, H_3PO_4 (0.05M) (Brendlen *et al.*, 2003)	Brushite
Shanghai Rebone Biomaterials Co (CN)	Rebone	Powder: TetCP, DCP; Solution: H_2O (Liu *et al.*, 1997)	Apatite
Skeletal Kinetics (US)	Callos™	Composition: α-TCP, $CaCO_3$, MCPM; Solution: sodium silicate(Constantz, 2002)	Apatite
	Callos Inject™	Composition: α-tricalcium phosphate and unknown compounds (likely to be close to that of Callos™)[1]	Apatite
	OsteoVation EX Inject	Probably similar to "Callos Inject™" (Product produced by S.K. but sold by OsteoMed)	Apatite
Stryker (US) Leibinger (GER)	BoneSource	Powder: TetCP (73%), DCPD (27%); Solution: H_2O, mixture of Na_2HPO_4 and NaH_2PO_4 (Brown and Chow, 1985; Brown and Chow, 1983; Chow, 1991)	Apatite
	HydroSet™	Powder: TetCP, DCPD, trisodium citrate; Solution: H_2O, polyvynilpyrrolidone, sodium phosphate (Hannink *et al.*, 2008)	Apatite
Synthes (US)	Norian® SRS Norian® CRS	Powder: α-TCP (85%), $CaCO_3$ (12%) MCPM (3%) ; Solution: H_2O, Na_2HPO_4 (Constantz *et al.*, 1995; Fernandez *et al.*, 1998)	Apatite
	Norian® SRS Fast Set Putty Norian® CRS Fast Set Putty	Composition: could not be found (likely to be close to that of Norian SRS/CRS)[1]	Apatite
	Norian Drillable	Composition: calcium phosphate powder, bioresorbable fibers and sodium hyaluronate solution	Apatite
	chronOS™ Inject	Powder: β-TCP (73%), MCPM (21%), $MgHPO_4 \cdot 3H_2O$ (5%), $MgSO_4$ (<1%), $Na_2H_2P_2O_7$ (<1%); Solution: H_2O, sodium hyaluronate (0.5%) (Bohner *et al.*, 2003)	Brushite
Teknimed (FR)	Cementek®	Powder: α-TCP, TetCP, Na Glycerophosphate; Solution: H_2O, $Ca(OH)_2$, H_3PO_4 (S. Goncalves, Teknimed, private communication)	Apatite
	Cementek® LV	Powder: α-TCP, TetCP, Na Glycerophosphate, dimethylsiloxane; Solution: H_2O, $Ca(OH)_2$, H_3PO_4 (S. Goncalves, Teknimed, private communication)	Apatite
Wright Medical (US)	MIIG™ 115	Powder: CSH; Solution: Saline (Turner *et al.*, 2003)	Gypsum
	MIIG® X3	Composition: CSH; Solution: Sterile water (contains also traces of an accelerant)	Gypsum
	MIIG® X3 High-Visc	Composition: CSH; Solution: Sterile water (less than in MIIG® X3; contains also traces of an accelerant)	Gypsum
	Pro-Dense®	Composition: 75% CSH, 25% brushite and granular β-TCP	Gypsum

[1]Not found in the literature or on the web

[2]FDA website (http://www.fda.gov/search.ht) – Classification product code: MQV

Table 3. list of some non-setting non-allogenic pastes with indication of producer, product name, composition and form (pre-mixed or to be mixed). Denominations: BCP = biphasic calcium phosphate (composite between HA and β-TCP); CMC = carboxymethylcellulose; HPMC: hydroxypropylmethylcellulose.

Producer	Product name	Composition	Form
ApaTech (UK)	Actifuse™	HA, polymer and aqueous solution[1]	Pre-mixed
	Actifuse™ Shape Actifuse™ ABX	Silicon-substituted calcium phosphate and polymer	Pre-mixed
Baxter (US)	TricOs T TricOs	BCP (60% HA, 40% β-TCP) granules and Tissucol (fibrin glue)[1]	To be mixed
Berkeley Advanced Biomaterials	Bi-Ostetic Putty	Non-disclosed[1]	Not disclosed[1]
BioForm (US)	"Calcium hydroxylapatite implant"	HA powder embedded in a mixture of glycerine, water, and CMC[1]	Pre-mixed
Biomatlante (FR)	MBCP Gel®	BCP granules (60% HA, 40% β-TCP; 0.08-0.2mm) and 2% HPMC (Boix *et al.*, 2006; Gauthier *et al.*, 2005)	Pre-mixed
	Hydr'Os	BCP granules (60% HA, 40% β-TCP; micro and nanoparticles) and saline solution (Biomatlante, private communication)	Pre-mixed
Degradable solutions (CH)	easy graft™	β-TCP or BCP granules (0.45-1.00mm) coated with 10 μm PLGA, N-methyl-2-pyrrolydone (K. Ruffieux, private communication)	To be mixed
Dentsply (US)	Pepgen P-15® flow	Hydroxyapatite (0.25-0.42mm), P-15 peptide and aqueous sodium hyaluronate solution (product brochure)	To be mixed
DePuy Spine (US)	Healos® Fx	HA (20-30%) and collagen[1]	To be mixed
Fluidinova (P)	nanoXIM TCP	β-TCP (5 or 15%) and water (company website)	Pre-mixed
	nanoXIM HA	HA (5, 15, 30, or 40%) and water (company website)	Pre-mixed
Integra LifeSciences (US)	Mozaik Osteoconductive Scaffold	β-TCP (80%) and type 1 collagen (20%)	To be mixed
Mathys Ltd (CH)	Ceros® Putty / cyclOS® Putty	β-TCP granules (0.125-0.71mm; 94%) and recombinant sodium hyaluronate powder (6%)	To be mixed
Medtronic (US)	Mastergraft®	BCP (85% HA, 15% β-TCP) and bovine collagen[1]	To be mixed
NovaBone (US)	NovaBone® Putty	Bioglass and synthetic binder[1]	Pre-mixed
Orthovita (US)	Vitoss Flow	Contains at least bioactive glass and saline solution (or blood marrow aspirate, or blood)[1]	To be mixed
	Vitoss Pack	Contains at least bioactive glass and saline solution (or blood marrow aspirate, or blood)[1]	To be mixed
Osartis / AAP (GER)	Ostim®	Nanocrystalline HA (35%) and water (65%) (Laschke *et al.*, 2007)	Pre-mixed
Smith & Nephew (US)	JAX CS	CSD granules and an aqueous solution (http://global.smith-nephew.com/us/JAX_CS_OVERVIEW_7221.htm)	To be mixed
	JAX TCP	β-TCP granules and an aqueous solution of 1.75% CMC and 10% glycerol (Clarke *et al.*, 2007)	To be mixed
Stryker (US)	Calstrux™	β-TCP granules and CMC[1]	To be mixed
Teknimed (FR)	Nanogel	Nanocrystalline HA (100-200nm) (30%) and water (70%) (S. Goncalves, private communication)	Pre-mixed
Therics (US)	Therigraft™ Putty	β-TCP granules and polymer[1]	Pre-mixed
Zimmer (US)	Collagraft	BCP granules (65% HA, 35% β-TCP; 0.5-1.0 mm), bovine collagen, and bone marrow aspirate (Bucholz, 2002)	To be mixed

[1]FDA website (http://www.fda.gov/search.html) – Classification product code: MQV

static mixer (Chow and Takagi, 2002; Lemaitre *et al.*, 2003). This approach has more time-constraints than ready-to-use putties, but since mixing only occurs in the cannula (= static mixer), the cannula can be changed, for example after cement hardening, and more cement can be injected. However, such an approach is difficult to apply to highly-viscous pastes (leads to syringe plugging). Moreover, it has only been described for brushite CPCs which are much less used than apatite CPCs (Lemaitre *et al.*, 2003) or for a cement paste that contains a non-aqueous water-miscible liquid (Chow and Takagi, 2002). The last and third approach to mix the paste is to combine the powder(s) with the liquid(s) just before use. This approach is more cumbersome, but also more versatile than the two other ones since it allows the addition of various components (e.g. drug solution, platelet-rich plasma, etc…). Moreover, it is generally easier to have the liquid and the solid component as single components during production (e.g., for sterilization – see hereafter). However, as a change of cement composition affects the setting reaction, the modification of the composition by the user is not recommended with setting pastes. To conclude, mixing is not only defined by the possibilities offered by the chemical nature of the product (non-setting or setting paste, composition), but also by the versatility that the producers would like to offer to their costumers, as well as the limitations set by the product manufacturing.

Once implanted, bone graft substitute must be visible by radiological means. Even though ceramic-based products are much more visible than putties based on demineralised bone, there is a need to improve their radiological contrast. So far, three main strategies have been used: (i) add a non-resorbable radiopaque additive such as Tungsten, Tantalum, Bismuth oxide or Barium sulphate particles; (ii) add a resorbable radiopaque additive such as Strontium carbonate as in "Kyphos" product (Table

2); (iii) add an iodine-based liquid as in "Cerament" product (Table 2). Unfortunately, none of these strategies is very convincing. First, the presence of non-resorbable particles in a resorbable material may eventually lead to the release of billions of particles. Second, strontium carbonate is hardly more radiopaque than calcium phosphates. Finally, iodine-based liquid may cause allergic reactions days after product implantation, with potentially dramatic consequences.

Hardening (for Cements)

A very important handling property of cements is their hardening rate (= setting rate) because it directly affects the clinical procedure. Specifically, a too early setting reaction limits the period during which the surgeon can apply the cement, whereas a too late setting reaction prevents the surgeon to close the defect and hence extent the overall procedure duration. Generally, the setting rate is characterized by measuring the setting time, i.e. the time it takes to reach a certain mechanical stability, either using a Gillmore needle (ASTM, 1999) or the Vicat test (ASTM, 2002). Unfortunately, the setting time is just one point along the curve relating compressive strength and reaction time. In other words, the setting time does not describe the shape of the curve, e.g. the presence of initial lag, or stepwise versus steady increase. Therefore, various authors have made efforts to not only better characterize the setting reaction but also better understand the factors affecting it (Bohner *et al.*, 2006b; Fukase *et al.*, 1990; Fulmer and Brown, 1990; Ginebra *et al.*, 1999; Hofmann *et al.*, 2006).

A large number of strategies exist to modify the hardening rate of cements, for example, changing the particle size of the reagents, adding a nucleating phase, or dissolving adequate additives (accelerators or retarders) into the mixing solution (Bohner, 2007). So, it is easy to modify the cement composition to reach a setting time that is clinically relevant, typically close to 10min. Unfortunately, it is more difficult to simultaneously control the initial rate of the reaction and the overall cement reaction (for example to shorten the overall duration of the setting reaction). Therefore, there is potential for improvement and efforts have been focused towards this goal. For instance, it was recently shown that a simple thermal treatment at 500ºC could extend the initial part of the setting reaction from a few minutes to a few hours hence providing a potential approach to better control the setting reaction (Bohner *et al.*, 2009). In 2007, Brunner *et al*. demonstrated that nanosized amorphous calcium phosphate particles could be used to produce cements reacting within an hour (Brunner *et al.*, 2007).

Mechanical Properties

The compressive strength of CPCs and calcium sulphate cements is generally one of the properties presented in scientific publications. It is also often put forward by commercial organizations. Unfortunately, these values are close to be meaningless due to the inherent brittleness of ceramics: the indication of a mean compressive strength of e.g., 50 MPa measured on perfectly-shaped and perfectly-prepared samples (e.g., under vibrations and pressure) does not inform the reader with which probability this cement will fail in situ under a cyclic load of e.g. 10 MPa. The comparison of the compressive strength of the cementious bone substitute with that of cancellous bone is not very helpful either because cancellous bone is much less brittle than ceramic cements. In fact, the reader should get additional information regarding the strength distribution of the cementious material (so-called Weibull distribution (Morgan and Dauskardt, 2003)). Moreover, since loads always contain shear or tensile components, the tensile or the shear properties should also be measured (Charriere *et al.*, 2001). Typically, as result of the material brittleness, the tensile strength is one order of magnitude lower than the compressive strength. Last but not least, loads are generally cyclic, which means that fatigue properties and fracture mechanics are aspects that should also be addressed (Gisep *et al.*, 2004; Morgan and Dauskardt, 2003). When all these measurements are considered, it becomes very clear that CPCs and calcium sulphate cements can only be applied in non-load-bearing applications. The poor mechanical properties of CPCs explain why CPCs are not even performing well in applications where low load-bearing properties are required, for example in bone augmentation (Blattert *et al.*, 2006; Libicher *et al.*, 2006; Libicher *et al.*, 2005; Maestretti *et al.*, 2007; Nakano *et al.*, 2005; Nakano *et al.*, 2002).

Another important aspect to consider when looking at the mechanical properties of cementious bone substitutes is that the mechanical properties may vary quite extensively upon implantation. For example, since gypsum and brushite are soluble in physiological conditions, the mechanical properties of these materials rapidly decrease upon implantation (Ikenaga *et al.*, 1998). This spontaneous dissolution is also the reason why these materials are often combined with less soluble bone substitute such as β-TCP or HA (Hanker et al., 1986; Ohura *et al.*, 1996; Sato *et al.*, 1998).

Biological Properties

Since none of the bone substitutes proposed so far in the scientific world are load-bearing or even close to be load-bearing, the main strategy presently used to repair bone defects is to use a bone substitute that is rapidly resorbed and replaced by new mature bone. To reach this goal, not only the chemistry but also the geometry of the bone substitute has to be optimized (Bohner, 2000; Bohner and Baumgart, 2004). For example, it is particularly important to use a bone substitute that can be easily invaded by cells and blood vessels. For that purpose, the bone substitute must have a fully interconnected porous structure with diameters of pores and pore interconnections larger than about 50μm (Bohner and Baumgart, 2004; Karageorgiou and Kaplan, 2005; Lu *et al.*, 1999; von Doernberg *et al.*, 2006). CPCs are highly porous materials but do not contain

such macropores (here defined as pores with a diameter larger than 50μm). To remedy to this problem, CPC pastes have been combined with highly soluble solids (Barralet *et al.*, 2002; Fernandez *et al.*, 2005; Takagi and Chow, 2001; Xu *et al.*, 2001), hydrophobic liquids (Bohner, 2001), and gas bubbles (Almirall *et al.*, 2004; del Valle *et al.*, 2007; Ginebra *et al.*, 2007; Sarda *et al.*, 2002). Unfortunately, the as-generated macropores are generally not interconnected which limits the extent of this strategy. Another approach to obtain a macroporous pasty bone substitute is to combine granules with a hydrogel, for example sodium alginate (Klein *et al.*, 1987), dextran (Chan *et al.*, 2002), sodium hyaluronate (Chazono *et al.*, 2004), and hydroxypropylmethyl cellulose (Dupraz *et al.*, 1998; Grimandi *et al.*, 1998). Since the solid content of hydrogels is generally very low (a few percents), cells can easily penetrate the hydrogel-filled macroporous gaps present between granules. The size of the macroporous gaps is controlled by the hydrogel fraction and by the granule size distribution.

Another important aspect that should be addressed here is related to the size of the ceramic particles present in the bone substitute paste. It is indeed known that the *in vivo* response of particular bone substitutes is a function of their dimension and amount (Frank *et al.*, 1991; Pioletti *et al.*, 2000). For example, Evans and Clarke-Smith (Evans and Clarke-Smith, 1991) observed that "only (HA) particles smaller than about 5 μm are able to cause damage". So, the biological responses of pastes consisting of loose nano or microsized particles might differ from pastes consisting of mm-large particles. Since it is only recently that products consisting of densely packed but loose particles have been introduced (Tadic and Epple, 2004), there is presently too little *in vivo* data to really assess the potential risks or benefits associated with loose nano-or microsized particles. Therefore, caution is required when designing a ceramic bone substitute consisting of loose nano- or microsized particles.

In the last decades, there has been a trend towards the use of highly resorbable bone substitutes. Whereas some of these materials, such as β-TCP, are resorbed by cells, other such as gypsum and brushite are resorbed by simple dissolution. For example, at equilibrium, a solution obtained by dissolving gypsum in water has a calcium concentration roughly 10 times higher than that of serum. Also, serum does not contain sulphate ions. As a result, gypsum dissolution in serum is expected to proceed fairly rapidly. Consequently, gypsum dissolves more rapidly than bone grows leading to the appearance of fibrous tissue in the defect centre (Urban *et al.*, 2003). Compared to gypsum, brushite is one order of magnitude less soluble, but it is still slightly soluble in physiological conditions. So, brushite cements have been shown to rapidly loose their mechanical strength (Ikenaga *et al.*, 1998) and to transform in their centre into an apatite (Bohner *et al.*, 2003; Constantz *et al.*, 1998; Penel *et al.*, 1999). Also, a fibrous gap is observed between ingrowing bone front and resorbing cement front (Apelt *et al.*, 2004; Theiss *et al.*, 2005). However, this gap disappears when only apatite remains in the CPC block.

In the original CPC formulation proposed by Brown and Chow (Brown and Chow, 1985; Brown and Chow, 1983), no fibrous gap is observed between cement and bone, but physico-chemical changes also occur within the CPC paste due to the fact that the latter formulation sets in basic conditions (Greish and Brown, 2003): apatite precipitates *in vivo*, hence leading to denser and stronger cements (Ishikawa *et al.*, 1994). As apatite is a basic compound, the precipitation of apatite acidifies the surrounding medium. This is not a problem for an inherently basic cement such as the one proposed by Brown and Chow (Brown and Chow, 1985; Brown and Chow, 1983), but has been thought to provoke negative *in vivo* reactions when large amounts of brushite cements are implanted (Bohner *et al.*, 2003; Flautre *et al.*, 1999). To conclude, the use of fast resorbable cement may lead to a rapid transformation of a bone defect into mature bone, but bears the risk of negative biological reactions and/or a too fast disappearance leaving an empty defect.

Industrial Design

When the composition of a bone graft substitute has been optimized to achieve adequate handling, physico-chemical, and biological properties, other problems might arise and render the project unfeasible: non-availability of raw materials, poor product shelf-life, or difficulty to sterilize the product. These aspects are discussed in the next paragraphs.

Whereas in certain fields the specifications of a raw material can be freely chosen, restrictions often exist in the bone graft substitute field due to a small market size. For example, when purchasing calcium phosphates, it is of interest to get a high purity. Unfortunately, most commercially-available calcium phosphates contain impurities in concentrations high enough to cause problems (e.g. Mg content in powders used for α-TCP synthesis (Carrodeguas *et al.*, 2008; Enderle *et al.*, 2005)). Whereas the problem might not be too stringent for ceramic raw materials, more problems might arise to purchase polymeric rheological additives. Currently, hyaluronates (acid or salt) have the highest availability among pharma-grade polymer additives. However, hyaluronates are generally sold as unsterile powders and sterilization is complicated (ultra-filtration), particularly for highly-concentrated solutions (> 3%). Furthermore, when hyaluronates are sold as solution (e.g. for aesthetic surgery, arthrosis, ophtalmology), the concentration is generally too low (typically < 1%) and the volume is either too large (200-300mL) or too small (0.2-1.0mL). Finally, when all criteria are fulfilled, hyaluronates producers might not be willing to sell the material due to a too small need from the bone graft substitute producers.

Once the product is packaged, it must be sterilized. Unfortunately, polymers and ceramics may require different sterilization methods. For example, most polymers lose their integrity during gamma-sterilization and sometimes also during autoclaving. Alternatively, ceramics are often unstable during autoclaving (e.g. CSD).

As a result, it might be impossible to find a way to sterilize a ceramic-polymer paste. In the latter case, the only solution is to purchase sterile products and to mix them under aseptic conditions. Unfortunately, as many products cannot be bought sterile and cannot be easily sterilized (e.g., sodium hyaluronate), it might be impossible to produce a product according to its initial design. Instead of offering a one-component product, pre-mixed and ready to be injected to their costumers, companies might have to sell a two-component product that has to be mixed in the operating room.

Once packaged and sterilized, the product must be stable during storage, i.e. during the so-called shelf-life. Obviously, wet pastes are more likely to be unstable than dry mixtures. For example, calcium phosphates may dissolve and precipitate in the solution, leading to a change of the mean crystal/particle size or even to the formation of agglomerates. Since the rheological properties of a paste (e.g. injectability) depend on the mean particle size, rheological properties may be completely altered. Even the stability of dry mixtures is not a trivial problem. For example, Gbureck *et al*. (Gbureck *et al.*, 2005a) showed that extensive mixing of the dry components of a brushite CPC markedly decreased its shelf-life.

Future Trends

The last decade has experienced a tremendous change of the bone graft substitute market due to rapidly raising sales: whereas most companies sold only granules and blocks in the 1980s and 1990s, practically all major companies are now offering cements and putties. Furthermore, there is a clear trend towards a specialization of the products: companies are now designing products for specific clinical indications. In other words, the bone graft substitute market has reached a critical size. Since the sales, at least in number, are still rising, this trend will go on in the future.

A particularly strong and recent trend is the introduction of non-setting pastes or putties. Presently, there are as many non-setting pastes as cements. Since their production is often less tricky than that of cements (no need to provide a paste with always the same setting time), and their biological response is often better, it is very likely that there will soon be more commercial formulations of non-setting pastes than cements. Interestingly, academic research is very limited in this field.

Another important trend in the future will be the improvement of the biological properties of bone substitutes, the aim being to transform a bone defect into new mature bone as fast as possible. This implies that the focus will be set on resorbable materials that possess an open-porous structure allowing cells to invade the structure. Another potential focus could be set on osteoinductive ceramics (Habibovic and de Groot, 2007). A number of authors have indeed observed that ceramic bone graft substitutes implanted under the skin or in muscles are filled or coated with bone over time. However, despite very intensive research, there is only a poor understanding of the mechanisms leading to osteoinduction, and as a result, it is not possible at the moment to design an osteoinductive ceramic.

A last trend is to add minute amounts of foreign ions into ceramic bone graft substitutes to improve their biological behaviour. Most efforts have been set on Si, but other ions have been looked at such as Mg, Na, Sr, or Zn (Bigi *et al.*, 1997; Ergun *et al.*, 2002; Gibson *et al.*, 1999; LeGeros *et al.*, 1989; Saint-Jean *et al.*, 2005; Yoshida *et al.*, 2006). Even though effects can be expected, strong scientific evidence is still missing, partly because it is difficult to incorporate foreign ions without modifying other ceramic properties (e.g., solubility, grain size, pore size), and partly because it is difficult to synthesize truly-pure ceramics. As a result, it is always difficult or even impossible to know whether the change of biological reaction is due to the release of the investigated ions or to a different factor. A way out of this problem might be to load or coat the bone graft substitutes with soluble salts of the considered ions (Barralet *et al.*, 2009).

Acknowledgment

Grateful thanks to Y. Bruderer for her help in identifying new commercial products

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