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Factors influencing the compressive strength of an injectable calcium sulfate–hydroxyapatite cement

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A biphasic injectable bone substitute, suitable for filling bone defects, that sets in the body, based on calcium sulfate and hydroxyapatite (HA), is presented. For applications in bone defects the compressive strength is important to assure support of the defect site during loading when the patient is weight bearing. To control the strength, the influence of four different factors; the liquid-to-powder (L/P) ratio, the HA particle morphology, the HA content and the amount of accelerator, were investigated.

α-Calcium sulfate hemihydrate (CSH) and four different HA powders (three sintered and one spray-dried) were used. All differed in size and morphology. CSH and each HA powder were mixed together with distilled water to form the bone substitute. An accelerator, in form of calcium sulfate dihydrate, was added to the powder phase to obtain an adequate setting time. Cylindrical specimens were compression tested.

A lower L/P-ratio gave stronger cement, but was more difficult to inject. The shape and the morphology of the HA particles influenced the strength, and reducing the amount of HA increased the strength. The amount of accelerator (calcium sulfate dihydrate) had no influence.

Introduction

For more than a century, research has been carried out to find a suitable material to repair or replace bone segments. Autogenous bone, in the form of predominantly trabecular bone, still remains the material of choice for bone grafting today. Autografts are clearly osteogenic, but there is a limited supply of this type of bone graft, especially in children. The need of a second surgical site to harvest the graft gives the patient additional surgical trauma. To avoid this second operation site, allografts are used instead of autografts. Allografts, however, demonstrate lower osteogenic capacity and new bone formation may occur more slowly, but the most important drawback is the risk for infections mainly through transfer of viruses such as hepatitis and HIV viruses [1]. To reduce or eliminate the need for bone grafting substantial effort has been invested into finding a suitable artificial bone substitute.

It has been shown by various authors [2–6] that injectable calcium phosphates could be used as bone substitutes because of their bioactive properties. Most of the injectable calcium phosphate cements on the market transform to hydroxyapatite (HA) during the setting reaction. HA is especially suitable for implantation since it is chemically close to the calcium phosphate that is the mineral phase of bone. This similarity encourages the bone to accept the material as its own [4, 7] with minimal adverse reactions [8]. The main drawback using apatitic cements is the relatively slow resorption rate of HA, especially dense HA; it may remain in the body for more than 10 years [9].

Calcium sulfate, also known as plaster of Paris, is a fast resorbing material that has been used to fill bone defects during the whole 20th century. The use of calcium sulfate was first reported by Dreesmann [10] from the Trendelenburg clinic in 1892 and then later by Peltier [11] in the United States, who gained extensive clinical experience from the 1950s to the 1970s. Calcium sulfate has shown excellent biocompatibility and in 1980 Coetzee [12] concluded that it was a good bone graft substitute, comparable to autograft, in defects in the skull and facial bone. Calcium sulfate has caused concern due to its rapid resorption, before the bone tissue has had the time to grow into the defect, and thus has been replaced by hydroxyapatite as filling material for bone defects. However, recently more interest has been shown towards the use of calcium sulfate in orthopaedic applications. Nadkarni *et al.* [13] reported that composites of calcium sulfate with calcium phosphate can be formulated to resorb at controled rates. A composite containing 35% of weight of calcium phosphate showed that 33.33% of the implant volume was resorbed after 3 weeks in femoral metaphyseal defects in rabbits, while after 6 weeks the resorption was 51.28%. Sato *et al.* [14], also showed that

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osteoconductivity can be obtained with a calcium sulfate bone graft substitute combined with hydroxyapatite.

In this study we allowed calcium sulfate hemihydrate [$CaSO_4 \cdot 1/2H_2O$; CSH] to react with water to form calcium sulfate dihydrate [$CaSO_4 \cdot 2H_2O$; CSD] Equation 1, which has a microstructure in form of entangled needles [15].

$$CaSO_4 \cdot 1/2H_2O + 3/2H_2O \rightarrow CaSO_4 \cdot 2H_2O \qquad (1)$$

HA particles were added to the CSH before mixing with water and they became incorporated in the CSD-structure formed during the setting reaction. HA does not react with calcium sulfate.

The mechanical properties are low compared to apatitic cements, but reaching a compressive strength comparable to that of trabecular bone [16] would enable this bone substitute to be used clinically. Since bone is mainly subjected to compressive forces the compressive strength of the cement is important to provide good support for filled defects. Therefore, the aim of the study was to investigate how the formulation influenced the compressive strength of this new injectable calcium sulfate-HA bone substitute. The factors in question were the liquid-to-powder (L/P) ratio, the HA particle morphology, the amount of HA particles in the cement and the amount of accelerator.

Materials and methods

Materials

α-Calcium sulfate hemihydrate [$CaSO_4 \cdot 1/2H_2O$; CSH] and four different hydroxyapatite powders [$Ca_{10}(PO_4)_6(OH)_2$; HA], were used. Three of them, HA-A, HA-B and HA-C, had been sintered. All displayed differences in shape and size distribution. The fourth HA-powder (HA-S-D) had been precipitated from a solution with a supersaturation of calcium and phosphate and then spray-dried in air.

Characterization of the HAs

The four HAs were examined with scanning electron microscopy (SEM, Philips 515). All the samples were gold coated to make them electrically conductive. The analysis was performed using an accelerating voltage of 20 kV.

Size distribution analysis was performed in air using laser diffraction equipment consisting of a detection component (HELOS, Sympatec GmbH, Germany) and an injection component (RODOS, Sympatec GmbH, Germany) specialized for dry dispersion.

The particle surface area was measured with BET (Micromeritics ASAP 2400, Ciab Chemical Instrument, Atlanta, USA), using N_2 [17]. As a pre-treatment, the samples were put in test-tubes, under vacuum, and degasified for 24 h at 400 °C. They were weighted and then placed in the test station where they were cooled with liquid $N_2 (T = -196\,°C)$ on the outside of the test tubes. The test-tubes were replaced under vacuum in case any air had leaked in during the weighing. The measurements of the surface area was then performed by stepwise adding of 100% N_2 (gas) until a relative pressure of 0.99 was obtained. By measuring the volume of N_2 needed for each step, the adsorption of N_2 in the powder surface could be calculated and thus the surface area.

Mixing cements

Each HA was mixed with CSH and then with distilled water to obtain pastes. To make the cement set within a desired time (< 15 min) an accelerator consisting of ground calcium sulfate dihydrate [$CaSO_4 \cdot 2H_2O$; CSD] was added to the powder phase. Mixing was performed by hand and at atmospheric pressure.

Compression testing

After mixing, the cement was injected into Teflon-molds and placed in 100% relative humidity (above water in a desiccator) for 48 h. The obtained samples were cylindrical ($\phi = 4$ mm, $h = 8$ mm). Mechanical testing was performed using an Universal Testing Machine (Instron 8511.20). The samples were placed between self-levelling plates and compressed at $1\,\text{mm}\,\text{min}^{-1}$ until failure. Statistic analysis was performed using one-way ANOVA for general comparison and Mann-Whitney U- and Student *t*-test to compare individual groups. Four studies were performed to investigate how certain factors influenced the compressive strength of the new calcium sulfate-HA cement. For each test a minimum of six samples were used.

Influence of HA morphology

Four materials were tested, each containing a different HA morphology. All powders contained 50% by weight (wt %) HA and 50 wt % CSH. For the cements containing the three sintered HAs (-A, -B and -C) a liquid-to-powder (L/P) ratio of $0.3\,\text{mL}\,\text{g}^{-1}$ was used while for the cement containing HA-S-D (cement-S-D) the L/P ratio was $0.6\,\text{ml}\,\text{g}^{-1}$ to obtain a paste with similar viscosity.

Influence of the amount of sintered HA in the cement

Two different HA contents, 40 and 50 wt %, were investigated. Two sintered forms of HA; HA-B and HA-C, were used for the study. The L/P ratios used for cement-B and cement-C were 0.32 and $0.3\,\text{mL}\,\text{g}^{-1}$, respectively.

Influence of the liquid-to-powder (L/P) ratio

The influence of the L/P ratio on the compressive strength of the cement was determined for the cement containing 40 wt % HA-A (cement-A) and 0.2 wt % accelerator. Six L/P ratios, in $\text{mL}\,\text{g}^{-1}$, were tested; 0.375, 0.400, 0.425, 0.450, 0.475 and 0.500.

Influence of the amount of accelerator added to the cement

Cement-A and -B were tested with different amounts of accelerator to investigate its influence on the compressive strength at 40 wt % HA. Four different amounts of accelerator; 0.2; 0.4; 0.8; 1.2 wt % were added to the powder phase of cement-A at a L/P ratio of 0.4 mL g^{-1}. For cement-B two different amounts of accelerator were added; 0 and 0.5 wt % at a L/P ratio of 0.32 mL g^{-1}.

Results

Influence of HA morphology

The differences in the particle surface between the HAs are shown in Table I. HA-S-D had a much larger surface area than the others. The sintered HA particles were denser and smaller (Tables I and II; Fig. 1, HA-A, -B and -C) than the spray-dried (Fig. 1, HA-S-D). This increase in surface area means that it had a greater ability to adsorb water and therefore needed more water to form an injectable cement. This water adsorption explains the difference in L/P ratio $(\mathrm{L/P} = 0.6\,\mathrm{mL\,g^{-1}})$ between this cement and cements-A, -B and -C $(\mathrm{L/P} = 0.3\,\mathrm{ml\,g^{-1}})$ to obtain similar viscosity.

SEM pictures showed a greater interconnectivity between the CSD-crystals in the final cement when the sintered HAs were incorporated (Fig. 2, cement-A, -B and -C) than when the spray-dried HA was used (Fig. 2, cement-S-D). In cement-A (Fig. 2, cement-A) it was hard to distinguish the CSD-crystals from the HA-particles. The major part of the CSD-crystals were short, almost cubical, and only some regions showed crystals with the characteristic needle shape. The needle-shaped crystals had a length of 10–20 μm while the short crystals were about 2–5 μm long. Cement-B (Fig. 2, cement-B) had a different microstructure with long flat CSD-crystals and larger HA-particles (6–8 μm) possible to distinguish from the CSD-matrix. The CSD-crystals laid together, oriented in one direction, forming blocks. The sizes of the crystals were about 10–15 μm in length. In cement-C (Fig. 2, cement-C) the CSD-crystals were shorter than in cement-B, about 4–6 μm in length, but they were less flat and spread in every direction forming a matrix of well interconnected crystals. The HA-particles presented in the cement had almost the same size as the thickness of the CSD-crystals and, therefore, they were hard to identify. The cement containing the spray-dried HA (Fig. 2, cement-S-D) showed a different microstructure compared to the other cements. The two phases were easily observed, the HA-particles as large balls inside the phase of interconnected CSD-needles. These needles were thinner but had the same length as seen in the other cements (4–8 μm). Statistical analysis by one-way ANOVA showed that there was a significant difference in compressive strength between the four cements $(p < 0.001)$ (Fig. 3). Cement-S-D had a compressive strength of 8.8 ± 1.4 MPa while the three sintered HA cements, -A, -B and -C, attained 11.8 ± 3.9, 18.7 ± 2.9 and 25.5 ± 3.9 MPa, respectively. Further statistical analysis by Fisher's pairwise comparisons showed that each cement was significantly different to the others, except cement-A compared with cement-S-D.

TABLE I Particle surface characteristics of the four HA-powders

Hydroxyapatite	BET surface area/m^2 g^{-1}	Pore volume/cm^{3}g^{-1} adsorption	Pore size/nm adsorption
HA-A	1.9174	0.005784	13.81336
HA-B	3.3100	0.011555	15.56273
HA-C	1.1611	0.003230	13.19013
HA-S-D	69.8597	0.379563	23.56516

TABLE II Particle size distribution of the four HA-powders

Hydroxyapatite	$D_{0.1}/\mu m$	$D_{0.5}/\mu m$	$D_{0.9}/\mu m$
HA-A	0.33	1.98	7.87
HA-B	0.18	1.06	3.46
HA-C	1.50	4.24	12.48
HA-S-D	1.65	12.27	29.71

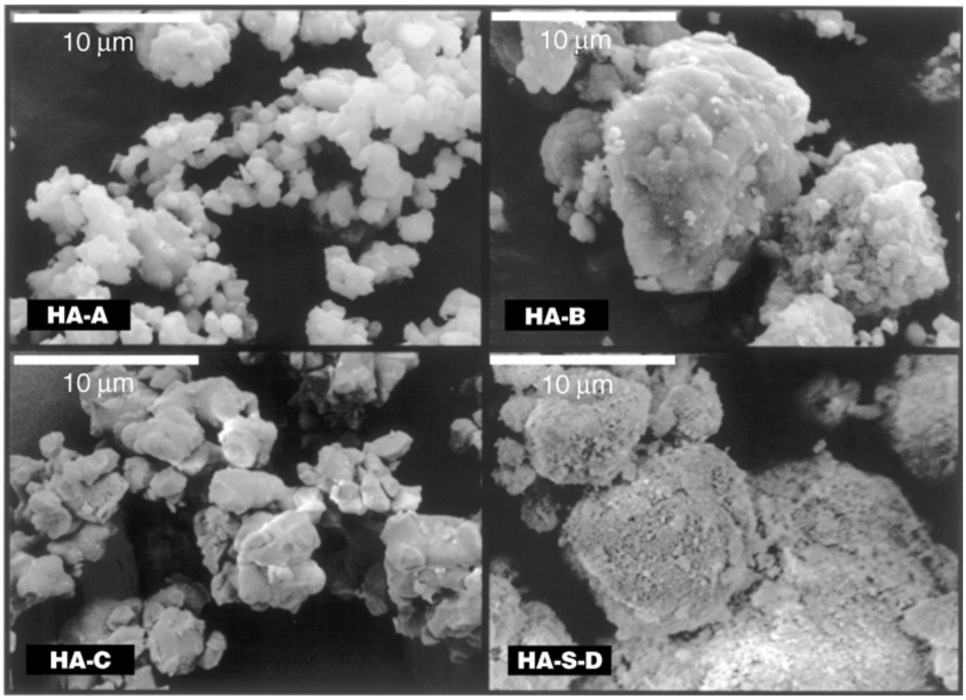

Figure 1 Scanning electron micrographs of the four forms of HA used (marker bars = 10 μm).

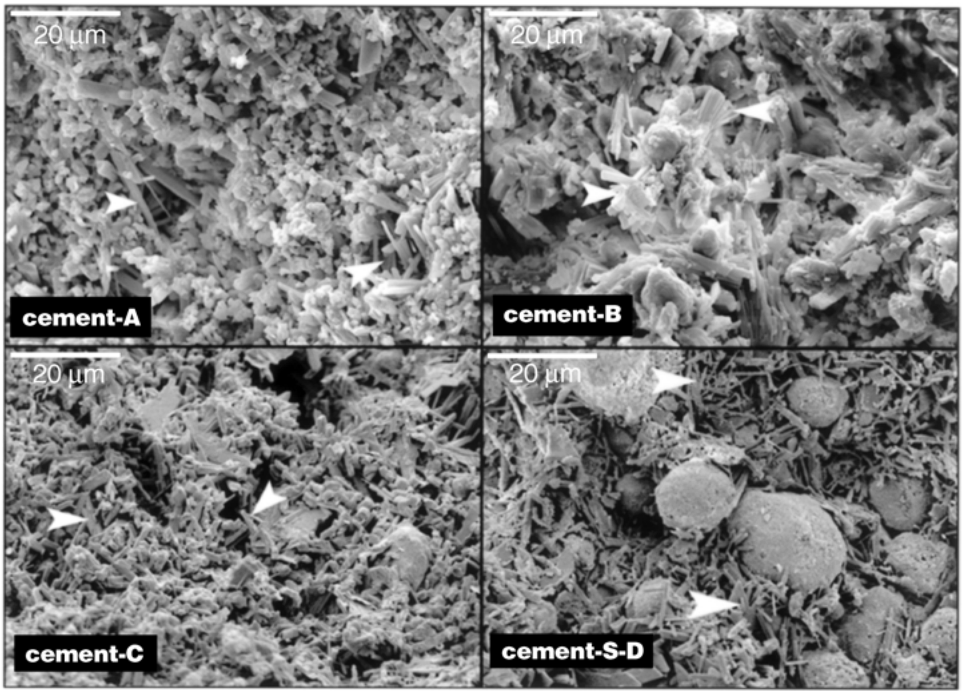

Figure 2 Fracture surfaces of the four materials tested, containing 50 wt % of HA (marker bars = 20 μm). The white arrows show the CSD-crystals.

Influence of the amount of sintered HA in the cement

For cement-B and -C higher strengths were obtained for the cements containing 40 wt % HA than those containing 50 wt % HA (Fig. 4). In the case of cement-B the increase in strength between the cement containing 50 wt % HA (18.7 ± 2.9 MPa) and the one containing 40 wt % HA (25.7 ± 3.5 MPa) was 37.4% ($p < 0.001$). In the case of cement-C the increase in strength was 21.6% between the cement containing 50 wt % HA (25.5 ± 3.9 MPa) and the one containing 40 wt % HA (31.0 ± 6.1 MPa) ($p = 0.022$).

Influence of the L/P ratio

The strength of the cement decreased linearly ($y = 56.38 – 84.33x$) when more water was added (Fig. 5). The compressive strength for cement-A decreased by 38.9%, from 25.7 ± 3.2 MPa for L/P $= 0.375\,\mathrm{mL\,g^{-1}}$ to 15.7 ± 1.2 MPa for L/P $= 0.5\,\mathrm{mL\,g^{-1}}$. A significant difference in strength was seen between the lowest and the highest L/P in the range ($p < 0.001$).

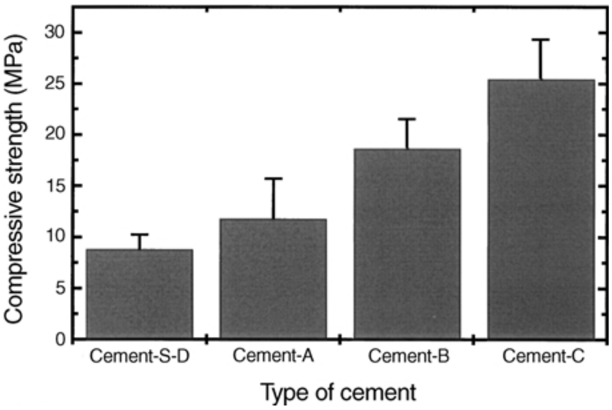

Figure 3 Influence of HA morphology on the compressive strength.

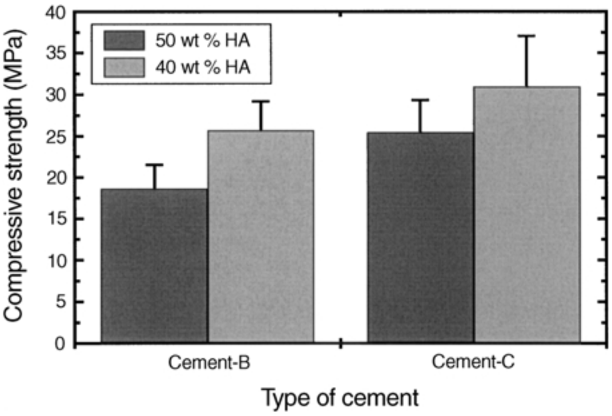

Figure 4 Influence of the amount of HA on the compressive strength.

Influence of the amount of accelerator added to the cement

The compressive strengths obtained for cement-A at the four different amounts of accelerator (0.2; 0.4; 0.8; 1.2 wt %) were all between 15 and 18.5 MPa. The strength was not significantly influenced by the amount

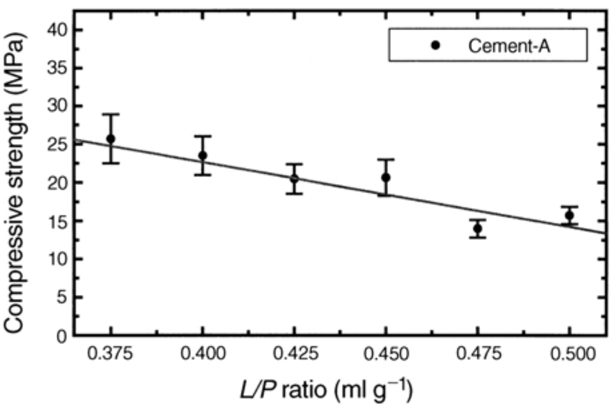

Figure 5 Influence of the L/P ratio on the compressive strength.

of accelerator ($p > 0.05$). In cement-B similar behavior was seen. For the two amounts of accelerator, 0 and 0.5 wt %, the materials showed compressive strengths of 25.7 ± 3.5 and 24.8 ± 4.5 MPa, respectively ($p > 0.05$).

Discussion

The strength of the CSD-matrix decreases as the L/P ratio is increased, i.e. the content of liquid in the material. The decrease in strength is caused by an increase of the porosity in the CSD, thus reducing its strength [15]. There was a linear relation between the decrease in strength and the increase in L/P ratio in the tested range.

The form of HA is another factor of importance for the compressive strength. The lowest strength was obtained for the bone substitute based on spray-dried HA. The spray-dried HA had a porous surface, which adsorbed water and therefore the L/P ratio had to be increased to keep viscosity sufficiently low for the material to be injectable and more water increases the porosity thus reducing the strength. Furthermore, the spray-dried particles were larger and this fact, combined with the increased water content, made the CSD-crystals grow separately with less entanglement than occurred when using the sintered HAs. The more the CSD-crystals could entangle with each other the stronger material obtained [15]. In the scanning electron micrograph of the cement containing spray-dried HA (Fig. 2, cement-S-D), the HA particles appear as obviously separate particles within the cement, unlike the cements based on the sintered HAs, where it is difficult to distinguish between the CSD-crystals and the HA. Furthermore, the CSD-crystals observed in the cement-S-D seem thinner compared to the other cements, which also may combine to a weaker strength. There were also differences in strength between the three sintered HAs. We could show no relation between particle size [18], particle specific surface area, L/P ratio and the compressive strength. However, some differences were seen in the crystal structure of the CSD-phase. The CSD-crystals in cement-A (Fig. 2, cement-A) were short and, therefore, less entangled. Yet, there were some regions with longer crystals but that made the material inhomogeneous. The crystals have had the space to grow longer in regions with large pores in the cement, which could explain the low strength. Cement-B (Fig. 2, cement-B) showed clusters of CSD-crystals oriented in the same direction. The crystals were longer than in cement-A but wider and flatter, forming more a plate than a needle. Longer crystals would enhance entanglement and thus increase the strength. In cement-C (Fig. 2, cement-C) the CSD-crystals were homogeneously spread in all directions, which would increase the strength even further as compared to the block-like structure of cement-B.

The amount of HA particles in the cement also influenced the strength. Two different amounts of HA, 40 and 50 wt % were chosen, based on the osteoconductivity shown in two previous studies. Sato *et al.* [14] showed that osteoconductivity, which is a requirement for the cement, could be obtained with 50 wt % HA and Härtter [19] showed that osteoconductivity was not obtained with less than 20 wt % HA. The idea was to optimize the amount of HA for compressive strength while still showing osteoconductivity. Cement-B and -C showed that the strength increased when less HA was added. Since there are no reaction or chemical bonding between calcium sulfate and HA in the cement, the two phases (CSD and HA) are only held together by the CSD-matrix. HA is only mechanically incorporated in the crystal structure and does not assist in binding the material together. Thus, by increasing the HA content there will be less CSD in the material to keep it together and, as a result, the strength will decrease. Additionally, the HA is acting as a brittle inclusion in a brittle matrix, thus the more filler (HA) and the larger the filler particle size the worse the effect on the mechanical properties of the composite [20].

The strength was not influenced by the amount of accelerator. The accelerator, which consisted of the same material as the cement matrix (CSD), acted as an initiator for crystal growth [21]. The CSD-crystals started to grow from the accelerator nuclei and at the end of the setting it was impossible to distinguish the accelerator from the matrix. The fact that the amount of accelerator does not change the strength is an advantage since it is added only to modify the setting time.

To obtain a relatively strong material, which is still mixable and can be injected or molded, it is necessary to find a compromise between the injectability and the mechanical strength. The challenge is to find the lowest water content while the material still could be mixed to an injectable bone substitute.

This material could not be used in weight bearing cortical bone, but should be applicable as a filler for contained cavities in trabecular bone [5]. The material may also have sufficient strength to significantly decrease the time for which an external cast or brace is necessary and/or to enhance fracture healing. If this is possible, the patient's mobility is not limited to the same extent as it would be if a cast was necessary for a long period of time [22]. This decreases the risks for stiffness and reduced mobility for the patient after operation.

The use of this substance for vertebroplasty, or other compression fractures of trabecular bone, seems possible as injection is easy to perform and setting temperature is below body temperature.

Conclusions

The factor that affected the strength the most was the amount of water added to the powders, as increasing the water produced a weaker cement because of increased porosity.

The shape and morphology of HA was also important for the strength of the bone substitute. The materials containing sintered HA showed higher compressive strengths than the one containing spray-dried HA.

Increasing the HA content decreased the compressive strength of all the cements tested.

The accelerator content had no influence on the compressive strength.

A setting calcium sulfate combined with particulate HA could act as an injectable bone graft material and may be a potentially useful biomaterial with orthopaedic and dental applications, in particular for defect filling in trabecular bone.

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References

1. P. FRAYSSINET, J. FAGES, G. BONEL and N. ROUQUET, *Eur. J. Orthop. Surg. Traumatol.* **8** (1998) 17.
2. B. BAI, L. M. JAZRAWI, F. J. KUMMER and J. M. SPIVAK, *Spine* **24** (1999) 1521.
3. B. R. CONSTANTZ, I. C. ISON, M. T. FULMER, R. D. POSER, S. T. SMITH, M. VANWAGONER, J. ROSS, S. A. GOLDSTEIN, J. B. JUPITER and D. I. ROSENTHAL, *Science* **267** (1995) 1796.
4. P. D. COSTANTINO, C. D. FRIEDMAN, K. JONES, L. C. CHOW and G. A. SISSON, *Plast. Reconstr. Surg.* **90** (1992) 174.
5. T. KITSUGI, T. YAMAMURO, T. NAKAMURA, S. KOTANI, T. KOKUBO and H. TAKEUCHI, *Biomaterials* **14** (1993) 216.
6. F. C. M. DRIESSENS, M. G. BOLTONG, O. BERMUDEZ and J. A. PLANELL, *J. Mater. Sci: Mater. Med.* **4** (1993) 503.
7. J.-S. SUN, H.-C. LIU, W. H.-S. CHANG, J. LI, F.-H. LIN and H.-C. TAI, *J. Biomed. Mater. Res.* **39** (1998) 390.
8. J. S. WANG, S. GOODMAN and P. ASPENBERG, *Clin. Orthop.* **304** (1994) 272.
9. S. N. KHAN, E. TOMIN and J. M. LANE, *Orthop. Clinics North America* **31** (2000) 389.
10. H. DREESMANN, *Beitr. Klin. Chir.* **9** (1892) 804.
11. L. F. PELTIER, *Clin. Orthop.* **21** (1961) 1.
12. A. S. COETZEE, *Arch. Otolaryngol.* **106** (1980) 405.
13. P. P. NADKARNI, J. L. RICCI, J. R. PARSONS, M. HAWKINS, F. DIMAANO, D. PATEL, I. TARKIN and J. GARCIA, in ‘‘Sixth World Biomaterials Congress Transactions, Vol. I, Hawaii, May 2000’’, edited by A. F. von Recum and L. C. Lucas (Society for Biomaterials, Minneapolis, 2000) p. 113.
14. S. SATO, T. KOSHINO and T. SAITO, *Biomaterials* **19** (1998) 1895.
15. F. WIRSCHING, in ‘‘Ullmann’s Encyclopedia of Industrial Chemistry, Fifth, Completely Revised Edition, Vol. A4, Benzyl Alcohol to Calcium Sulfate’’, edited by W. Gerhartz, Y. S. Yamamoto, F. T. Campbell, R. Pfefferkorn and J. F. Rounsaville (VCH Verlagsgesellschaft mbH, Weinheim, 1985) p. 555.
16. F. C. M. DRIESSENS, J. A. PLANELL, M. G. BOLTONG, I. KHAIROUN and M. P. GINEBRA, *Proc. Inst. Mech. Eng. [H]* **212** (1998) 427.
17. C. A. BRUNAUER, P. H. EMMETT and E. TELLER, *J. Am. Chem. Soc.* **60** (1938) 309.
18. M. P. GINEBRA, E. FERNÁNDEZ, F. C. M. DRIESSENS, M. G. BOLTONG and J. A. PLANELL, *Bioceramics* **10** (1997) 481.
19. S. HÄRTTER, in ‘‘Experimentelle Untersuchungen zum Einfluß von Kalziumsulfat auf die Knochenheilung bei Kaninchen’’ (Fachverlag Köhler, Gießen, 1998).
20. R. RIEDEL (ed.), in ‘‘Handbook of Ceramic Hard Materials, Part I’’ (Wiley-VCH Verlag GmbH, Weinheim, 2000) p. 66.
21. A. J. LEWRY and J. WILLIAMSON, *J. Mater. Sci: Mater. Med.* **29** (1994) 5279.
22. P. KOPYLOV, K. RUNNQVIST, K. JONSSON and P. ASPENBERG, *Acta Orthop. Scand.* **70** (1999) 1.

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