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Premixed macroporous calcium phosphate cement scaffold

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Abstract

Calcium phosphate cement (CPC) sets in situ to form resorbable hydroxyapatite and is promising for orthopaedic applications. However, it requires on-site powder-liquid mixing during surgery, which prolongs surgical time and raises concerns of inhomogeneous mixing. The objective of this study was to develop a premixed CPC scaffold with macropores suitable for tissue ingrowth. To avoid the on-site powder-liquid mixing, the CPC paste was mixed in advance and did not set in storage; it set only after placement in a physiological solution. Using 30% and 40% mass fractions of mannitol porogen, the premixed CPC scaffold with fibers had flexural strength (mean $\pm$ sd; $n = 5$) of (3.9 ± 1.4) MPa and (1.8 ± 0.8) MPa, respectively. The scaffold porosity reached $(68.6 \pm 0.7)\%$ and $(74.7 \pm 1.2)\%$, respectively. Osteoblast cells colonized in the surface macropores of the scaffold and attached to the hydroxyapatite crystals. Cell viability values for the premixed CPC scaffold was not significantly different from that of a conventional non-premixed CPC known to be biocompatible ($P > 0.1$). In conclusion, using fast-dissolving porogen and slow-dissolving fibers, a premixed macroporous CPC scaffold was developed with strength approaching the reported strengths of sintered porous hydroxyapatite implants and cancellous bone, and non-cytotoxicity similar to a biocompatible non-premixed CPC.

Introduction

Hydroxyapatite is an important biomaterial because of its similarity to the apatitic mineral in natural bones [1–4]. However, sintered hydroxyapatite implants are available in pre-hardened forms, requiring the surgeon to carve the implant or modify the surgical site to fit the implant. This can lead to increases in bone loss, trauma and surgical time [5]. In contrast, calcium phosphate cements (CPC) can self-harden with easy manipulation and adaptation to the bone defect, and form bioresorbable hydroxyapatite [6–11]. Non-rigid, fast-setting and anti-washout CPC compositions have also developed to meet specific bone repair requirements [12,13].

The low strength of conventional CPC has limited its use to only non-stress-bearing applications [9–11]. Recently, CPC-absorbable fiber composites were developed that had much higher strengths than the unreinforced conventional CPC [14]. After fiber dissolution, long cylindrical macropores were created in CPC suitable for cell infiltration and tissue

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ingrowth [14]. The CPC-fiber composite supported osteoblast cell adhesion, proliferation and viability [14].

A common feature of these cements [6–14] is that there is a need to mix the powder with an aqueous liquid on the site in surgery. This increases surgical time, raises concerns about insufficient and inhomogeneous mixing, and may also cause unpredictable implant performance variations by different clinicians. Therefore, premixed CPC was recently developed to overcome these problems [15]. The CPC powder was mixed with a nonaqueous, but water-miscible, liquid in advance under well-controlled conditions. This waterfree paste did not harden in storage or in a syringe, because CPC hardens only when exposed to an aqueous environment. After this paste was placed in contact with a physiological solution, exchange of nonaqueous liquid–aqueous liquid occurred, leading to CPC hardening. But the premixed CPC had a setting time of longer than 1 h and a low mechanical strength [15]. A long setting time could cause problems clinically because of the cement's inability to support stresses within this time period. More recently, an improved premixed CPC formulation was developed; it exhibited rapid setting when immersed in a physiological solution, yielding a hardened cement with a higher strength [16]. However, the premixed CPC in these studies possessed no macropores suitable for cell infiltration and bone ingrowth.

Accordingly, one objective of the present study was to create macropores in the premixed CPC. Such macropores could improve cell migration into the scaffold and enhance implant fixation via new bone ingrowth. Since the premixed macroporous CPC scaffold was mechanically weak, the second objective was to impart strength and fracture resistance to the scaffold. These objectives were accomplished by incorporating both fast-dissolving pore-forming agents (porogen) and slow-dissolving fibers into the premixed CPC. The mechanical properties were investigated as a function of porogen amount and fiber reinforcement. In vitro cell culture was performed to examine the cytotoxicity of the premixed CPC and cell infiltration into the macropores.

Methods

Processing of macroporous premixed CPC scaffold

The CPC powder consisted of mass fractions of 73% tetracalcium phosphate (TTCP, $\text{Ca}_4[\text{PO}_4]_2\text{O}$) and 27% dicalcium phosphate anhydrous (DCPA, CaHPO_4), resulting in a TTCP:DCPA molar ratio of 1:1. The TTCP powder was synthesized from a solid-state reaction between equimolar amounts of CaHPO_4 and CaCO_3 (J. T. Baker Chemical, Phillipsburg, NJ), which were mixed and heated at 1500 °C for 6 h in a furnace (Model 51333, Lindberg, Watertown, WI). The TTCP was ground in a ball mill (Retsch PM4, Brinkman, NY) and sieved to obtain particles with sizes ranging from approximately 1 μm to 80 μm , with a median particle size of 17 μm . The DCPA powder was ground for 24 h and sieved to obtain particles with sizes ranging from approximately 0.4 μm to 3 μm , with median of 1 μm . The TTCP and DCPA powders were mixed to form the CPC powder.

Premixed CPC was formulated by: Premixed CPC = nonaqueous liquid + CPC powder + gelling agent + hardening accelerator. Glycerol (J. T. Baker Chemical) was used as the nonaqueous liquid following previous studies on premixed CPC without macropores [15,16]. The premixed powder consisted of 79.5% of the CPC powder mixed with 20% monocalcium phosphate monohydrate (MCPM, $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, Monsanto, St. Louis, MO) and 0.5% hydroxypropyl methylcellulose (HPMC) (Sigma, St. Louis, MO). HPMC was used as a gelling agent to improve the washout resistance of the cement [13]. MCPM was used as a hardening accelerator to reduce the paste hardening time [16].

Water-soluble mannitol has been used to produce macropores in conventional non-premixed CPC [17] because mannitol has the appropriate solubility and is non-toxic. Mannitol ($\text{CH}_2\text{OH}[\text{CHOH}]_4\text{CH}_2\text{OH}$, Sigma) was recrystallized in an ethanol/water solution at 50/50 by volume, and ground and sieved through openings of 500 μm (top sieve) and 300 μm (bottom sieve). The mannitol powder was combined with the premixed powder to form five mixtures at mannitol/(mannitol + premixed powder) mass fractions of 0%, 10%, 20%, 30%, and 40%. Each powder was mixed with the nonaqueous liquid to form a cohesive paste at a powder:liquid mass ratio was 4:1. The paste was filled into a mold of $3 \times 4 \times 25 \text{ mm}^3$, and sandwiched between two fritted porous glass slides (ACE Glass, Vineland, NJ). The assembly was immersed in a simulated physiological solution (1.15 mmol/L Ca, 1.2 mmol/L P, 133 mmol/L NaCl, 50 mmol/L Hepes, buffered to a pH of 7.4) in a humidifier with 100% relative humidity at 37 °C [12]. The use of the porous glass was to allow the nonaqueous liquid-water exchange, thereby causing the cement to harden. After 1 d, the hardened specimen was removed from the mold and immersed in the physiological solution for 2 d to dissolve the mannitol and form macropores in CPC [17].

Mechanical properties and porosity measurements

The specimens were fractured in three-point flexure on a computer-controlled Universal Testing Machine (model 5500R, Instron, Canton, MA). A span of 20 mm and a crosshead speed of 1 mm/min were used to measure the flexural strength and elastic modulus [18].

The halves of specimens from the flexural test were used to measure the density [18]. The specimens were dried in a vacuum oven at 60 °C for 24 h. The density was measured by the specimen mass divided by the specimen volume. As described in a previous study on conventional non-premixed CPC [18], the total porosity, P_{total} , can be obtained by:

$$P_{\text{total}} = (d_{\text{HA}} - d_{\text{measured}}) / d_{\text{HA}} \quad (1)$$

where d_{HA} is the density of fully-dense hydroxyapatite (3.14 g/cm³) [17], and d_{measured} is the measured density of the specimen with a specified mannitol mass fraction. The macroporosity from the dissolution of mannitol, P_{mannitol} , can be calculated by:

$$P_{\text{mannitol}} = 1 - (d_{\text{measured}} / d_{\text{measured-0\%}}) \quad (2)$$

where $d_{\text{measured-0\%}}$ is the measured density of the specimen with 0% mannitol.

Cell live/dead staining and viability

MC3T3-E1 mouse osteoblast-like cells (Riken, Hirosaka, Japan) were cultured at 37 °C and 100% humidity with 5% CO₂ (volume fraction) in α modified Eagle's minimum essential medium (Biowhittaker, Walkersville, MD) [19]. Four materials were tested: premixed CPC without mannitol, premixed CPC with 40% mannitol, conventional non-premixed CPC, and tissue culture polystyrene (TCPS) control. Conventional non-premixed CPC with water as the liquid was used as a control because of its known biocompatibility [11]. Specimens were hardened by immersion as described above and sterilized by autoclaving at 121 °C for 20 min. Cells were cultured on the surface of the specimens, so the three-dimensionally porous materials were biologically tested as two-dimensional materials. 50,000 osteoblast cells in 2 mL of medium were added to each well containing a cement specimen or to an empty well of TCPS and incubated for 1 d. Cells were stained for 5 min in 2 mL medium containing 2 $\mu\text{mol/L}$ calcein-AM and 2 $\mu\text{mol/L}$ ethidium homodimer-1 (Molecular Probes, Eugene, OR). The live cells were stained green and the dead cells red [20].

To quantify the cell viability, a flask of 80% confluent cells was passaged and cells were seeded into 24-well plates with 10,000 cells per well in 2 mL medium. Each cement specimen was immersed in a well with 2 mL of fresh medium without cells and extracted for 1 d to accumulate any possible harmful leach-out in the medium [16]. Then the medium from each well containing the cells was removed and replaced with the 2 mL of extraction medium. The cells were incubated in the extracts for 3 d and photographed with an inverted phase contrast microscope (Nikon TE300, Melville, NY). Cell viability was measured using the Wst-1 assay [21], which is a colorimetric assay of cellular dehydrogenase activity where absorbance at 450 nm is proportional to the amount of dehydrogenase activity in the cell. The absorbance was measured with a platereader (Perkin- Elmer, Gaithersburg, MD) [14].

Premixed CPC scaffold reinforcement

The above experiments showed that the premixed CPC scaffold was biocompatible but had a low mechanical strength. Therefore, an absorbable fiber (Vicryl, Ethicon, Somerville, NJ), shown to be biocompatible in a previous study on non-premixed CPC [14], was used to reinforce the premixed CPC. This braided fiber bundle had a diameter of about 322 μm , provided strength for about four weeks, and then dissolved and produced cylindrical macropores in a conventional non-premixed CPC [14]. The suture fiber was cut to filaments of 8 mm in length and randomly mixed into the premixed paste. A fiber volume fraction of 25% was used as calculated from the fiber density and specimen volume. The specimens were tested in flexure as described above.

A scanning electron microscope (SEM, JEOL 5300, Peabody, MA) was used to examine the specimens. Cells cultured for 1 d on cement specimens were rinsed with saline, fixed with 1% volume fraction of glutaraldehyde, subjected to graded alcohol dehydrations, rinsed with hexamethyldisilazane, and then sputtercoated with gold [14]. One standard deviation (sd) was used as the estimated standard uncertainty of the measurements. These values should not be compared with data obtained in other laboratories under different conditions. One-way and two-way ANOVA and Tukey's multiple comparison test were used at $p = 0.05$.

Results

Mechanical properties of premixed CPC scaffold without fibers

The flexural strength and elastic modulus of the premixed CPC scaffold without fibers are listed in Table 1 vs. mannitol mass fraction after 3 d immersion that dissolved the mannitol and created macropores. Flexural strength at 0% mannitol was significantly higher than those at 30% and 40% mannitol (Tukey's at $p = 0.05$). The elastic modulus showed a similar trend, with values at 0% mannitol, 30% mannitol, and 40% mannitol significantly different from each other ($p < 0.05$). The values at 20% and 30% mannitol were not significantly different from each other ($p > 0.1$).

Density and porosity

Increasing the mannitol fraction significantly decreased the specimen density and increased the porosity (oneway ANOVA; $p < 0.001$). In Fig. 1A, the straight line is a linear best fit: $d_{\text{measured}} = 1.61 - 2.06 M$, where M is the mannitol mass fraction (for example, 40% means that $M = 0.40$). The correlation coefficient $R = 0.99$. In Fig. 1B, the straight lines show: $P_{\text{total}} = 48.8 + 65.4 M$, and $P_{\text{mannitol}} = 131 M$, with $R = 0.99$ in both cases. At 30% and 40% mannitol, the total pore volume fraction (mean $\pm$ sd; $n = 5$) reached $(68.6 \pm 0.7)\%$ and $(74.7 \pm 1.2)\%$, respectively. The macroporosity in CPC from mannitol dissolution reached $(39.4 \pm 2.9)\%$ and $(51.3 \pm 3.4)\%$, respectively.

Live/dead cell staining

Cells cultured for 1 d are shown in Fig. 2, with live cells (green) on (A) TCPS control, (B) non-premixed CPC control (a known biocompatible cement without macropores), (C) premixed CPC (0% mannitol), and (D) premixed CPC scaffold (40% mannitol). Dead cells (red) were few on all materials, an example of which is shown in (E) for premixed CPC scaffold. The percent of live cells, equal to the number of live cells/(number of live cells + number of dead cells), was plotted in (F).

Culture in extracts and viability quantification

Cells cultured for 3 d in the extracts are shown in Fig. 3: (A) TCPS control, (B) non-premixed CPC control, (C) premixed CPC (0% mannitol), and (D) premixed CPC scaffold (40% mannitol). Cells cultured in the extracts of the premixed CPC and the premixed scaffold displayed a normal and polygonal morphology similar to the controls. The quantitative cell viability results are plotted in Fig. 3E (mean $\pm$ sd; $n = 5$). The absorbance at 450 nm was (0.58 ± 0.03) (arbitrary units) for the premixed CPC, not significantly different from (0.54 ± 0.09) of the non-premixed conventional CPC control or (0.64 ± 0.04) of the TCPS control ($p > 0.1$). The cell viability of the premixed CPC scaffold was (0.51 ± 0.08). Although it was significantly lower than the TCPS ($p < 0.05$), it was similar to the non-premixed conventional CPC control ($p > 0.1$). These results suggest that premixed CPC and premixed CPC scaffold were both as biocompatible as the nonpremixed CPC control.

Osteoblast-scaffold interactions

SEMs of the cells seeded onto the surface of the premixed CPC scaffold with 40% mannitol are shown in Fig. 4. Macropores were visible in (A). The pores were well-formed in the shapes of the dissolved mannitol crystals. The pore width ranged from about 100 μm to 200 μm , and the pore length ranged from about 200 μm to 400 μm . Pore “P” in Fig. 4A is shown at a higher magnification in (B), revealing that the osteoblast cells (O) had migrated into the macropore with anchorage on the pore bottom. The cells had sizes of about 20 μm to 40 μm , much smaller than the macropore size.

In Fig. 5A, the osteoblast cell “O” that colonized at the pore bottom was much smaller than the pore size, but its long cytoplasmic extensions (“E”) were established across the entire macropore, with the distal ends of the extensions anchoring on the pore walls. Fig. 5B shows that the cytoplasmic extensions attached to the small hydroxyapatite crystals (HA), enabling the anchorage of cells on the cement. In (C), cell-cell interactions and bridging were established between the four cells (“1” to “4”) anchored on the pore bottom. In (D), several cells migrated toward an opening at the pore bottom, or a pore interconnecting fenestration (indicated by “I”), and formed a three-dimensional cell web. Cells appeared to be using the hydroxyapatite (5A and B) and the neighboring cells (5C and D) as their support.

Fiber-reinforced strong scaffold

The flexural strength of the suture fiber-CPC composite scaffold was listed in Table 2. At each mannitol mass fraction, the flexural strength was significantly improved over the corresponding strength of CPC without fibers in Table 1 ($p < 0.05$). Elastic modulus of the fiber composite in Table 2 was significantly lower than the modulus without fibers (Table 1) at mannitol mass fractions of 0% and 10% ($p < 0.05$). At mannitol mass fractions of 20%, 30% or 40%, the fiber composite modulus was similar to the corresponding modulus of specimens without fibers ($p > 0.1$).

Discussion

Premixed macroporous CPC scaffold was developed for the first time. This was accomplished by using MCPM as a setting accelerator, HPMC as a gelling agent, glycerol as a nonaqueous liquid, mannitol as a porogen, and absorbable fibers for reinforcement. Premixed CPC eliminated the need for the on-site powder-liquid mixing during surgery. This would shorten surgical time, avoid insufficient or inhomogeneous mixing, and improve the implant performance by mixing the paste in advance under well-controlled conditions. In previous studies [1,3,22–24], macropores were shown to be useful in facilitating cell infiltration, tissue ingrowth and implant resorption. A macroporous implant is in the most need of strength in the early stages of implantation because once new bone grows into the macropores, the strength of the implant increases [4]. Premixed CPC scaffold without fibers had a low strength of 0.7 MPa (with 40% mannitol), and might fracture before bone ingrowth could occur. The absorbable fibers in the premixed CPC provided the needed early-strength. At mannitol mass fractions of 30% and 40%, the flexural strength of premixed CPC scaffold reached 3.9 MPa and 1.8 MPa, respectively. These values approached the lower end of the reported flexural strength of 2 MPa to 11 MPa for sintered porous hydroxyapatite implants and a tensile strength of about 3.5 MPa for cancellous bone [4]. Compared to sintered hydroxyapatite implants, premixed CPC requires no machining and can be molded to intimately adapt to complex cavity shapes. In addition, CPC forms bioresorbable hydroxyapatite that can be replaced by new bone in vivo [9–11].

CPC matrices with different strengths (Table 1) yielded CPC-fiber composites with different strengths (Table 2). It would be interesting to find the correlation between composite strength and matrix strength for the premixed CPC. Such a correlation could help guide composite preparation and predict performance based on the constituent properties. In a previous study [25], a semi-empirical equation was established that relates the CPC-fiber composite strength, S_c , to the fiber strength, S_f :

$$S_c = S_m + \alpha S_f \quad (3)$$

where S_m is the strength of CPC matrix without fibers, and α is a coefficient. In the previous study, the CPC matrix was held constant while S_f was varied by using different types of fibers [25]. In the present study, S_f was kept constant, while the CPC matrix was varied by changing the porosity via different mannitol fractions. The coefficient α in Eq. (3) should be proportional to S_m because if the CPC reached 100% porosity, then $S_m = 0$ and $S_c = 0$, thus requiring that $\alpha = 0$. This is because there would be no matrix holding and supporting the fibers. Although the fibers are strong, without a matrix there is no composite nor composite strength. Hence we assume $\alpha = \beta S_m$ to satisfy the condition that in Eq. (3), when $S_m = 0$, so should S_c , although S_f is not zero. Therefore, $S_c = S_m + \beta S_m S_f = S_m (1 + \beta S_f)$, and finally

$$S_c = \gamma S_m \quad (4)$$

where $\gamma = 1 + \beta S_f$. Figure 6 plots the CPC-mannitol-fiber composite strength S_c from Table 2 vs. the CPC matrix strength S_m from Table 1. The straight line is a linear best fit through the origin:

$$S_c = 1.88 S_m \quad (5)$$

with correlation coefficient $R = 0.97$. This equation shows that for the premixed CPC scaffold, the strength was increased by 1.88 times due to fiber reinforcement. It also shows that the composite strength increased linearly with increasing the matrix strength, which suggests the importance of not only using fibers, but also developing stronger matrix materials.

Previous studies showed that significant bone ingrowth into porous implants occurred in a few weeks to a couple of months [23,24,26]. Therefore, it would be desirable for the absorbable fibers to reinforce the CPC for a few weeks. In previous studies on non-premixed CPC-fiber composites [13,14], the fiber composite was shown to support osteoblast cell adhesion, proliferation and viability [14]. The fibers provided strength to CPC for about four weeks and then dissolved and produced macropores [13]. The rationale for using both mannitol and fibers in the premixed CPC was that, after several weeks, significant new bone would have grown into the macropores created by the mannitol, thus strengthening the scaffold. The fibers would then dissolve to create additional macropores for further bone ingrowth. Some clinical situations may require a longer reinforcement time than four weeks, hence different fibers should be tailored with controlled dissolution rates to match specific bone repair rates.

Among the components used in the premixed CPC, glycerol is known to be nontoxic and biocompatible, and has been used in beverages and chewing gum [27]. HPMC is biocompatible because it is a derivative of cellulose and is one of the common occurring polysaccharides [15]. MCPM is comprised of calcium, phosphate and water. Therefore, from the compositional point of view, it is of no surprise that the premixed CPC was found to be non-cytotoxic. The quantitative Wst-1 assay showed that cell viability was equivalent for all the tested materials. The cells developed cytoplasmic extensions ("E" in Fig. 5), thus enabling their anchorage onto the hydroxyapatite crystals in the premixed CPC. These capillary-like extensions are regions of the cell plasma membrane that contain a meshwork or bundles of actin-containing microfilaments which permit the movement of the migrating cells along a substratum [28]. They were also termed filopodia extensions and lamellipodia extensions [29]. The cells infiltrated into the surface macropores of the premixed scaffold and established cell-cell interactions. Further studies should investigate the effect of porosity (*e.g.*, premixed CPC without mannitol vs. premixed CPC with mannitol) on new bone growth and CPC resorption rates in animal models [10,11].

Summary

Premixed macroporous CPC scaffold was developed for the first time. It avoided the need for the on-site powder-liquid mixing which should shorten surgical time and allow the paste to be mixed thoroughly in advance under well-controlled conditions. Fast-soluble mannitol porogen created macropores in the premixed CPC, while fibers increased the scaffold strength to approach the reported strengths of sintered porous hydroxyapatite implants and cancellous bone. Compared to sintered hydroxyapatite, the advantages of the premixed CPC include moldability, intimate adaptation to complex bone cavities without machining, and formation of resorbable hydroxyapatite. The premixed CPC-fiber composite strength, S_c , was linearly correlated to the matrix strength S_m by: $S_c = 1.88 S_m$, suggesting the importance of using fibers as well as developing strong matrix materials. Osteoblast cells were able to infiltrate into the surface macropores of the premixed CPC, attach to hydroxyapatite crystals via cytoplasmic extensions, and establish cell-cell interactions. This premixed macroporous CPC scaffold may be useful in periodontal bone repair, mandibular and maxillary ridge augmentation, reconstruction of frontal sinus and craniofacial skeletal defects, and other moderate stress-bearing orthopedic applications.

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Fig. 1.

Density and porosity vs. mannitol mass fraction for premixed CPC scaffold. Each value is the mean of five measurements with the error bar showing one standard deviation (mean $\pm$ sd; $n = 5$). The straight lines are linear best fits, with $d_{\text{measured}} = 1.61 - 2.06 M$, $P_{\text{total}} = 48.8 + 65.4 M$, and $P_{\text{mannitol}} = 131 M$, and $R = 0.99$

Fig. 2. One-day cell culture, with live cells on (A) tissue culture polystyrene (TCPS, a biocompatible control), (B) non-premixed CPC control, (C) premixed CPC (0% mannitol), (D) premixed CPC scaffold (40% mannitol); and (E) dead cells on premixed CPC scaffold. (F) Percent of live cells = number of live cells/(number of live cells + number of dead cells). Each value is the mean of five measurements with the error bar showing one sd. Horizontal line indicates values that are not significantly different (Tukey's at $p = 0.05$). Each photo had the same magnification

Fig. 3. Three-day culture in extracts of: (A) TCPS control, (B) non-premixed CPC control, (C) premixed CPC without mannitol, and (D) premixed CPC scaffold (with 40% mannitol). The quantitative cell viability in (E) was the absorbance at 450 nm that was proportional to the dehydrogenase activity of the cells (arbitrary units). Each value is the mean of five measurements with the error bar showing one sd. Horizontal line indicates values that are not significantly different (Tukey's at $p = 0.05$). Each photo had the same magnification

Fig. 4. SEM micrographs of cells seeded on the surface of the premixed CPC scaffold (40% mannitol): (A) low magnification, and (B) higher magnification of the pore “P” in (A). In (B), O = osteoblast cells colonized inside a macropore

Fig. 5.

Features of cell-premixed CPC scaffold interactions. In (A), the cell colonized in a surface pore of the scaffold was much smaller than the pore size, but its long cytoplasmic extensions (“E”) were established across the entire macropore. In (B), cytoplasmic extension “E” enabled cell anchorage on small hydroxyapatite crystals “HA”. In (C), cell-cell interactions and bridging were established among four cells (“1” to “4”) anchored on the pore bottom. In (D), several cells migrated toward a pore interconnecting fenestration “I”

Fig. 6. Premixed CPC-fiber composite strength S_c (Table 2) vs. premixed CPC strength S_m without fibers (Table 1). The measured S_c and S_m for the specimens with the same mannitol fraction were fitted to Eq. (4) by linear regression to establish Eq. (5): $S_c = 1.88 S_m$, with $R = 0.97$. Each value was the mean of five measurements with the error bar showing one sd

Table 1
Mechanical Properties (mean $\pm$ sd; $n = 5$) of Premixed CPC Scaffold

Mannitol Mass%	0 %	10 %	20 %	30 %	40 %
Flexural Strength (MPa)	6.6 $\pm$ 1.8	3.8 $\pm$ 0.3	2.0 $\pm$ 0.2	2.1 $\pm$ 0.8	0.7 $\pm$ 0.2
Elastic Modulus (GPa)	3.92 $\pm$ 0.70	1.60 $\pm$ 0.19	0.94 $\pm$ 0.15	0.86 $\pm$ 0.22	0.32 $\pm$ 0.06

Table 2
Mechanical Properties (mean $\pm$ sd; $n = 5$) of Premixed CPC-Fiber Scaffold

Mannitol Mass %	0 %	10 %	20 %	30 %	40 %
Flexural Strength (MPa)	11.7 $\pm$ 1.5	7.3 $\pm$ 1.8	5.5 $\pm$ 1.9	3.9 $\pm$ 1.4	1.8 $\pm$ 0.8
Elastic Modulus (GPa)	1.58 $\pm$ 0.49	0.99 $\pm$ 0.27	0.72 $\pm$ 0.25	0.54 $\pm$ 0.21	0.37 $\pm$ 0.07