Injectable bioactive glass/biodegradable polymer composite for bone and cartilage reconstruction: Concept and experimental outcome with thermoplastic composites of poly(ε-caprolactone-co-D,L-lactide) and bioactive glass $S_{53}P_4$

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Injectable composites (Glepron) of particulate bioactive glass $S_{53}P_4$ (BAG) and Poly(ε-caprolactone-co-D,L-lactide) as thermoplastic carrier matrix were investigated as bone fillers in cancellous and cartilagineous subchondral bone defects in rabbits. Composites were injected as viscous liquid or mouldable paste. The glass granules of the composites resulted in good osteoconductivity and bone bonding that occurred initially at the interface between the glass and the host bone. The bone bioactivity index (BBI) indicating bone contacts between BAG and bone, as well as the bone coverage index (BCI) indicating bone ongrowth, correlated with the amount of glass in the composites. The indices were highest with 70 wt % of BAG, granule size 90–315 μm and did not improve by the addition of sucrose as *in situ* porosity creating agent in the composite or by using smaller (< 45 μm) glass granules. The percentage of new bone ingrowth into the composite with 70 wt % of BAG was 6–8% at 23 weeks. At the articular surface cartilage regeneration with chondroblasts and mature chondrocytes was often evident. The composites were osteoconductive and easy to handle with short setting time. They were biocompatible with low foreign body cellular reaction. Results indicate a suitable working concept as a filler bone substitute for subchondral cancellous bone defects.

Introduction

Two main factors have led to the interest focused on polymers in biomedical tissue reconstruction: the possibility of combining polymers with bioactive materials as solid implants [1, 2], and especially the possibility to use them as injectable materials in orthopaedics. The first polymer used was polymethylmethacrylate that was not fully injectable, but was used as mouldable cement [3]. During recent years studies have focused on *in situ* setting calcium phosphate cements [4], and methacrylate-based cements with hydroxyapatite granules or apatite–wollastonite glass-ceramic powder [5, 6]. Injectable composites of cellulose [7, 8] and poly(propylene fumarate) [9] have also been found to be osteoconductive. The well-known copolymers of ε-caprolactone and D,L-lactide have been shown to be suitable for biomedical applications [10, 11], however, there is only little experience of biodegradable aliphatic polyesters used in injectable form, particularly in living bone [12].

Properties of biomaterials related to bone reconstruction are illustrated in Fig. 1. An ideal bone substitute should have several properties [13, 14]; (1) non-toxicity, (2) bioactivity, (3) biocompatibility, (4) osteoconductivity and/or osteoinductivity, (5) sufficient mechanical properties, loading/weight-bearing capacity, (6) porosity allowing new bone ongrowth and ingrowth, (7) suitable degradation rate (although this is not mandatory), (8) convenient handling properties, (9) intraoperative mouldability and possibly (10) ductility permitting application by injection as a liquid or as a paste. In the concept above there may be a theoretical range where these properties are optimised. The working strategy in this study was to combine properties of the injectable biodegradable copolymer poly(ε-caprolactone-co-D,L-lactide) P(CL/DL-LA) and bioactive glass $S_{53}P_4$

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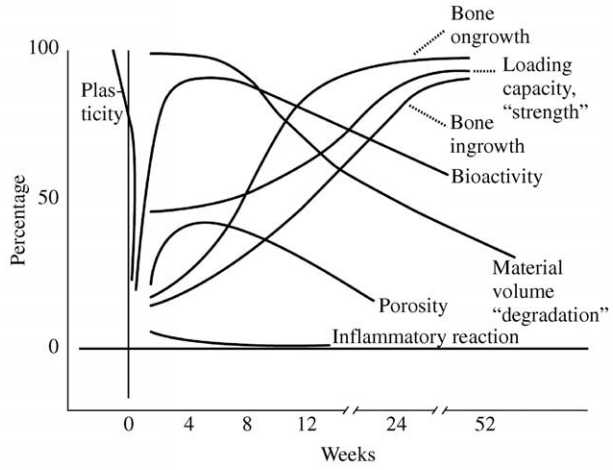

Figure 1 Theoretical model demonstrating properties and behaviour of an ideal biomaterial for bone tissue reconstruction as function of time. The concept includes bioactivity, bone ongrowth and ingrowth, and adjusted porosity being most important properties. The significance of the properties varies depending on whether the material is applied as injectable or solid, and whether it is used for trabecular or cortical bone reconstruction. Vertical axis (%) indicates relative activity of phenomena occurring in the implant–tissue incorporation. For example, the initial porosity of a bone substitute – about 30 vol % – may be especially suitable to allow bone ingrowth. Later on, the porosity decreases due to replacement of porosity by bone ingrowth.

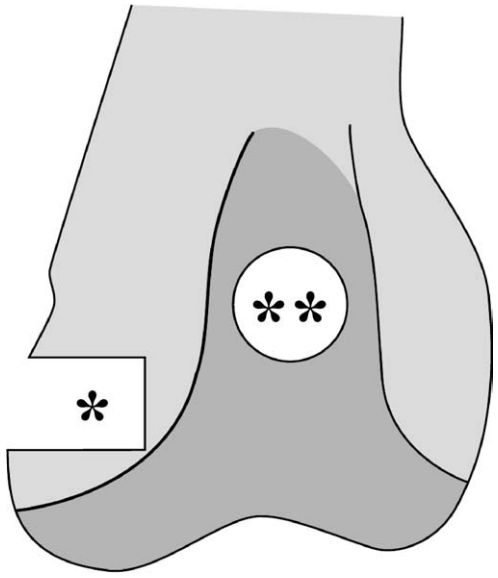

Figure 2 Surgical model showing the cavitary defects (diameter 6 mm, depth 5 mm) drilled in trabecular bone of the medial condyle (*) and through the intercondylar cartilage (**) in the knee joint of the distal femur of rabbit.

(BAG), and to find out suitable glass granule size and glass/copolymer ratio for bone reconstruction. The second aim was to study whether sucrose addition in the composite influence bone ingrowth by creating a tunnelling network porosity *in vivo*.

Materials and methods

Preparation of the glass/copolymer composites

Bioactive glass $S_{53}P_4$ (BAG) was made from SiO_2, Na_2CO_3, $CaCO_3$ and $CaHPO_42H_2O$ by melting them at 1360 °C for 3 h. Melted glass was then cooled down and crushed into granules, which were used in the study (Vivoxid Ltd, Turku, Finland). The weight percentage composition of the glass is SiO_2 53%, CaO 20%, Na_2O 23% and P_2O_5 4%. The copolymer used in the study was prepared in the laboratory of Polymer Technology at Helsinki University of Technology. Bioactive glass granules were blended homogenously with the copolymer in a Brabender W50EH batch mixer in 100 °C as described by Rich *et al*. [15]. The melting temperature of the copolymer was 50 °C, and monomer composition of the copolymer was (96/4) P(CL/DL-LA). Injectability of the composites is based on the thermal properties of the copolymer matrix and the composites remain injectable below 50 °C after heating. The liquid to solid transition in cooling is caused by the crystallisation of the copolymer matrix. For the *in vivo* experiments (Fig. 2) the thermoplastic composites, called Glepron, were injected at 47–50 °C (US Pat. No. 6 353 038/6.7.1998).

Composites were injected either as viscous liquid or mouldable paste. To be used as a liquid, the composites were packed into 1 ml syringes for the later use in plasticised form (Fig. 3(a)). To be used as a paste, the composite was melted and moulded in a tube with diameter of 5.5 mm, cooled down, and the surface of the solid composite rod was ground in order to expose the glass granules at its surface to improve initial bioactivity (Fig. 3(b)). Composites with different glass/copolymer wt % ratios and two different particle size ranges (90–315 and $< 45\,\mu m$) were used (Table I). Crystalline sucrose (S, particle size 300–1000 μm) was blended to one of the composites (BAG 50% + S 20%) as an *in situ* porosity creating agent. Copolymer without bioactive glass was used as a control material.

Biomechanics and handling properties

The compression test was performed by applying the International Standard for Orthopaedic Bone Cements ISO 5833/1 (Implants for surgery – Acrylic resin cements). Specimens for the strength test were cylinders, height 9.2 mm and diameter 4.6 mm. As the materials were elastic and did not fracture during the test, the compressive strength was determined at the 2.0% offset with crosshead speed 20.0 mm/min using a testing machine (model LRX, Lloyd Instruments Ltd., Fareham, England) connected with a PC computer program (Nexygen, Lloyd Instruments Ltd., Fareham, England). The two composites tested, BAG 70% liquid and BAG 50% + S 20%, had compressive strength of 7.7 ($\pm$ 0.5) MPa and Young's modulus of 153.7 ($\pm$ 26.5) MPa. Compressive strength of human cancellous bone reported in literature is 3–12 MPa [16].

The tested materials had good handling properties; they were ductile and in particular injectable below 50 °C provided that the CL/DL-LA ratio was 96–90/4–10. With lower ratios of CL/DL-LA the material was increasingly stiffer, and the viscosity impeded injection around 50 °C with a small diameter syringe. Using water-moistened operation gloves the composites were easy to handle and they did not adhere to the fingers.

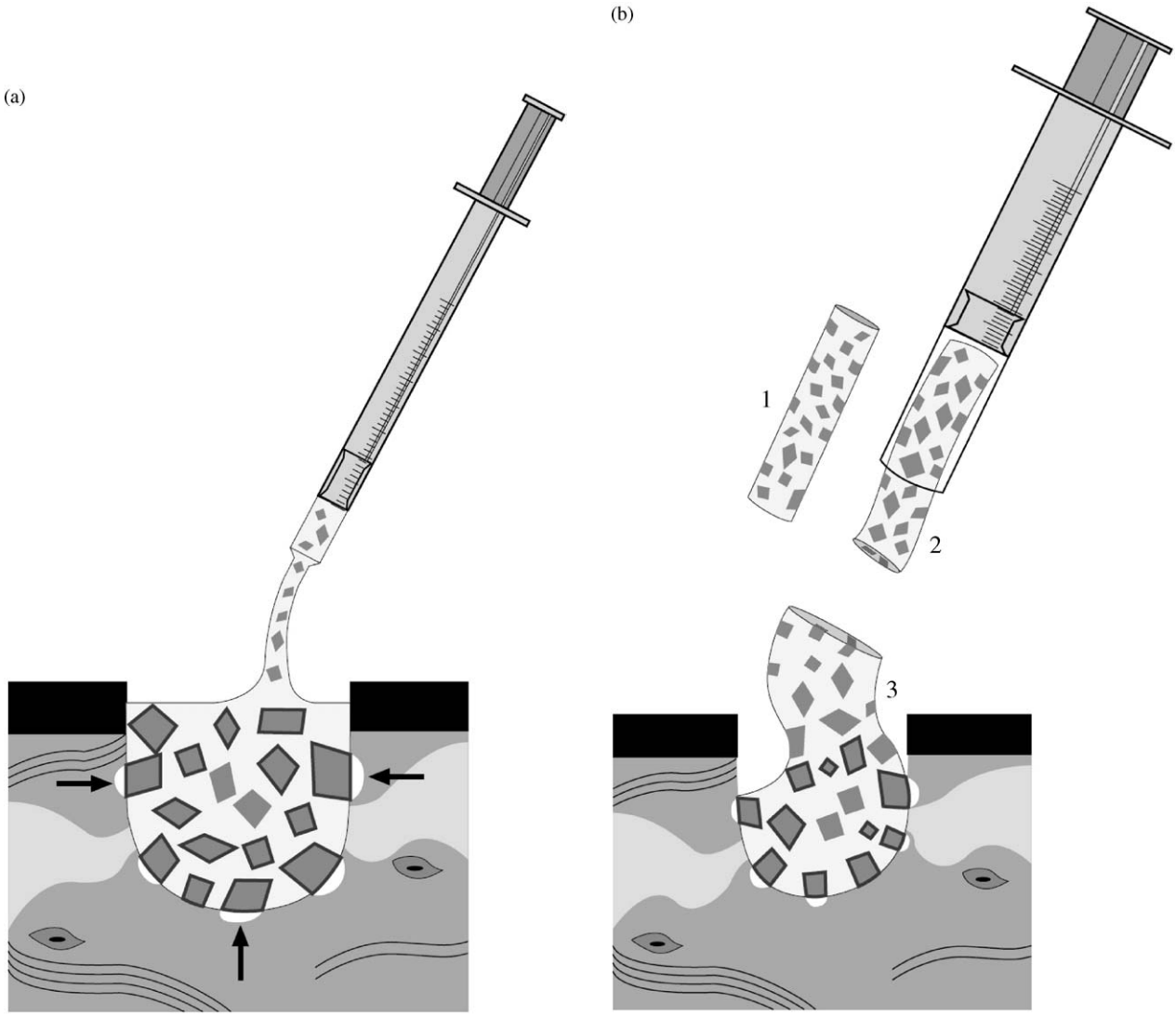

Figure 3 (a) Diagram illustrating the injection of the liquid form of thermoplastic composite of particulate BAG and P(CL/DL-LA) at 47–50 °C. Bone formation on the glass granules (arrows) will later lead to chemico-structural bonding and integration of the composite into the host bone. The surface reactivity of the glass is indicated by darkened lines at their edges. (b) Diagram illustrating the application of the paste form of the thermoplastic composite. The solid composite rod (1) with the glass granules exposed on the surface is heated to mouldable state (2) and injected into the bone defect (3).

TABLE I Bioactive glass $S_{53}P_4$(BAG)/P(CL/DL-LA) weight % ratios, glass granule size ranges and application form of the composites in cavitary bone defects of the distal femur in rabbits

BAG/P(CL/DL-LA)wt % ratio	Abbreviation	Glass granule size range (μm)	Form of application	Cavitary defects *N*
70/30	BAG 70% liquid	90–315	Liquid	25
70/30	BAG 70% paste	90–315	Paste	9
60/40	BAG 60%, small granules	< 45	Liquid	5
50/50 + Sucrose 20%	BAG 50% + S 20%	90–315	Liquid	8
40/60	BAG 40%	90–315	Liquid	4
Copolymer without BAG	Control	—	Liquid	6
				57
Unfilled defects	Empty	—	—	7
				64

Surgery, specimen preparation and analysis

Forty-six New Zealand white rabbits (females), 3.5–4.0 kg in weight, were used in the study. Protocol was approved by the Ethical Committee of the State Provincial Office of Western Finland, and national guidelines for laboratory animal care were followed. Surgery was performed according to the standards of aseptic orthopaedic treatment praxis. Anaesthesia was performed by intramuscular injections of midazolam 5 mg/ml, ketamine hydrochloride 50 mg/ml, and medetomidine hydrochloride 1 mg/ml. The operation areas were shaved and disinfected with chlorhexidine digluconate 5 mg/ml and covered with adhesive plastic cut-through operation skin barrier. A cavitary defect, 6 mm in diameter, 5 mm in depth ($N = 57$, Fig. 2) was drilled using sterile saline irrigation in the cancellous bone of

the intercondylar cartilage area $(N = 25)$ or in the medial condyle of the distal femur $(N = 32)$. Eleven rabbits had two defects. In addition, seven unfilled empty defects were used as controls of normal bone repair (Table I).

The composites were heated on hot-plate in sealed syringes and applied into the bone defects at 47–50 °C either as injectable viscous liquid or mouldable paste (Fig. 3). Paste form was carefully heated only to a mouldable state with intention to preserve the superficial glass granules exposed. The setting time of all composites was short, 20–30 s. Postoperative pain was treated for three days by intramuscular injections of buprenorfin hydrochloride 0.3 mg/ml. The animals were killed at four, eight, and 23 weeks by intravenous overdose of pentobarbitural 60 mg/ml. Samples for histology and histometry were obtained by dissecting the implant with surrounding bone. The specimens were fixed in 70% ethanol, dehydrated in ascending ethanol series and embedded in resin (Technovit, Heraeus-Kulzer GmbH, Wehrheim, Germany). 20 μm sections for light microscopy analysis were prepared by the cut-grind method developed for undecalcified hard tissue specimens [17]. Van Gieson, Masson-Goldner trichrome, toluidine blue and safranin stains were used. The resin embedded specimens and native composites were also analysed with scanning electron microscopy (SEM).

A computerised analysis system (Micro Scale TC, Digithrust Ltd, Royston, England) was used to count the number of glass–bone contacts and to measure the bone and connective tissue contact at the implant–tissue interface (Fig. 4).

The interface was quantified with two indices. The bone bioactivity index (BBI) was determined by using the formula

$$\frac{\text{number of glass–bone contacts}}{\text{defect length (cm)}} = \text{The bone bioactivity index}$$

The bone coverage index (BCI) was determined as the percentage amount of bone covering the implant at the interface.

The computerised analysis system was also used for the determination of new trabecular bone ingrowth into the composite implants (Fig. 4). This was expressed as area% compared to that of the trabeculae in the intact cancellous bone of the distal femur. Evaluation of the regeneration of cartilage on the composite surface was determined using the modified score (Table II) of Freed *et al*. [18] based on O'Driscoll *et al*. [19]. The score was modified by excluding the ''Absence of Degenerative Changes'' and by defining term ''Integrity'' to ''Integrity of tissue covering the implant''. Statistical analysis was performed with SPSS for Windows (Rel. 10.0.5, SPSS Inc, Chicago, Illinois, USA) using analysis of variance (ANOVA) and Tukey's test for *post hoc* analysis. P values lower than 0.02 were considered statistically significant.

TABLE II Histological scoring of cartilage formation covering the implants

Characteristics of the repair tissue	Score
A. Nature of the predominant tissue	
Hyaline articular cartilage	5
> 50% hyaline articular cartilage	4
50% hyaline cartilage/50% fibrocartilage	3
> 50% fibrocartilage	2
Fibrocartilage	0
B. Safranin-O staining of the matrix	
Normal staining	3
Moderate	2
Slight	1
None	0
C. Surface	
Smooth, intact surface	3
Superficial lamination	2
Slight disruption	1
Severe disruption	0
D. Integrity of tissue covering the implant	
51–100%	2
25–50%	1
< 25%	0
E. Thickness	
Equal to adjacent cartilage	2
50–99% of adjacent cartilage	1
0–49% of adjacent cartilage	0
F. Bonding to host tissue	
Bonded	2
Partially bonded	1
Not bonded	0
Maximum	17

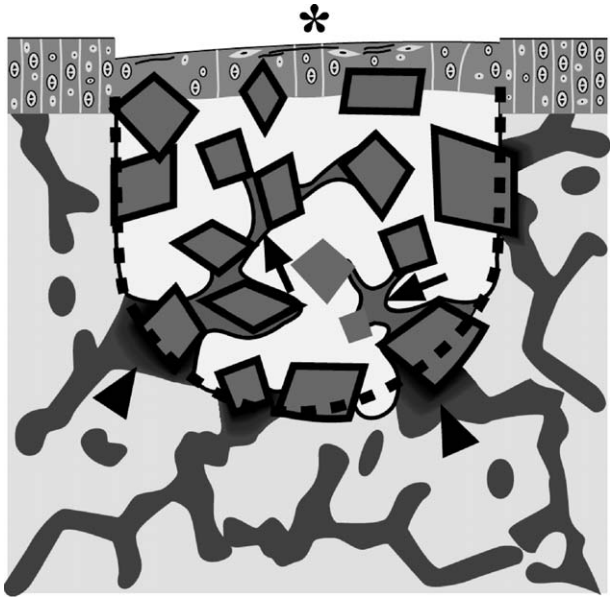

Figure 4 Schematic picture of the regeneration process in the subchondral bone defect filled with injected thermoplastic composite. Bone formation on the surface reaction layers of the bioactive glass granules (arrow heads) conduct the bone ingrowth into the composite (arrows). Cartilage formation (*) is illustrated covering the composite at the level of adjacent host cartilage. The dotted line indicates the implant-tissue interface that was used for the histometric measurements (BCI).

Results and discussion

SEM

SEM examination of the composite BAG 70% liquid revealed that at the time of injection the bioactive glass granules are homogenously embedded in the copolymer matrix (Fig. 5(a)), and the composite surface consists of a thin copolymer skin covering the granules. At four weeks after implantation direct bone growth onto the glass was

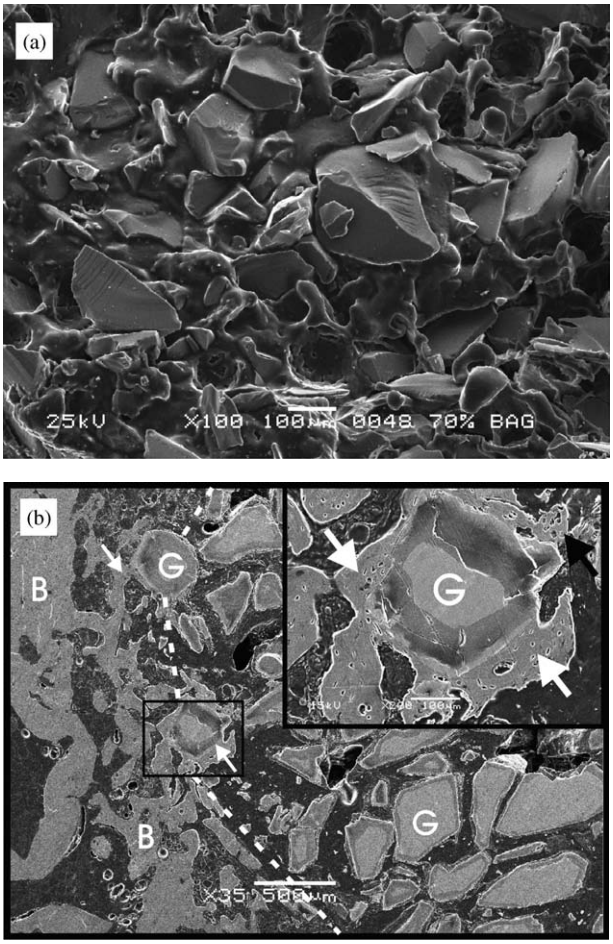

Figure 5 (a) SEM image of cross-section of the native composite BAG 70% liquid. Bioactive glass granules are homogenously embedded in the copolymer matrix. Image scale bar 100 µm. (b) SEM image illustrating BAG 70% paste at 23 weeks in cancellous bone defect. Trabecular host bone (B, on the left) bonds (small arrows) to bioactive glass granules (G) at the composite surface. Morphologically lamellar bone has grown into the composite mostly via the glass granules (thick arrows). The dotted line indicates implant-tissue interface. Image scale bar 500 µm. Insert rectangle scale bar 100 µm.

observed. Structural integrity confirmed bone bonding occurring initially via glass granules (Fig. 5(b)). Polymer skin bioresorption in the course of time was indicated also by an increase of BBI.

The implant–tissue interface – trabecular and cortical bone repair

Osteoconductive capacity of the tested materials was dependent predominantly on the glass copolymer ratio in the composites. In general, both indices correlated with the BAG content in the composites.

The BBI increased from eight to 23 weeks being significantly highest with BAG 70% liquid at 23 weeks (Fig. 6). BBI was lowest with BAG 40% with significant difference compared to BAG 70% liquid. Generally, tissue in contact with the glass granules differentiated into bone in the course of time (Fig. 7).

The BBI increased over the observation period (Fig. 8) being on average 88% at 23 weeks with BAG 70% liquid with significant difference compared to BAG 50% + S 20% (Table III). Neither the application as paste nor the sucrose addition increased BCI, on the contrary, value at

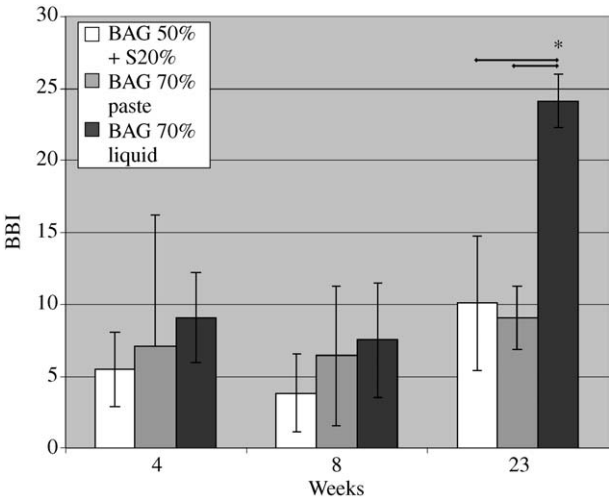

Figure 6 The BBI (average values and standard deviations) indicating the number of glass–bone contacts. BBI was significantly higher at 23 weeks ($* = p < 0.01$, Tukey's test) with BAG 70% liquid compared to paste form and sucrose addition groups.

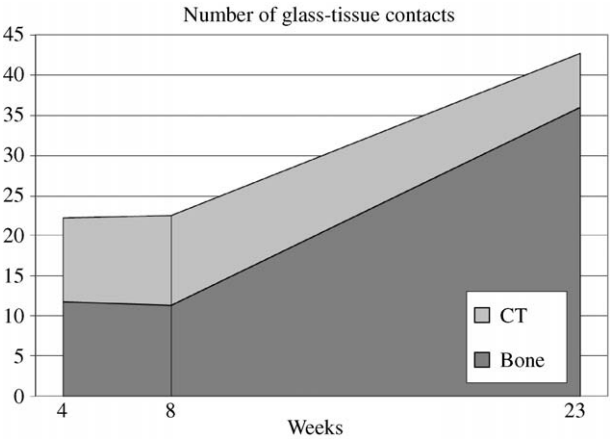

Figure 7 The average number of BAG granules in contact with bone and fibrous connective tissue (CT) in the composite BAG 70% liquid. The increase in the number of glass–tissue contacts indicate bone ingrowth and resorption of the composite. Proportion of bone increased and that of connective tissue decreased over the observation period.

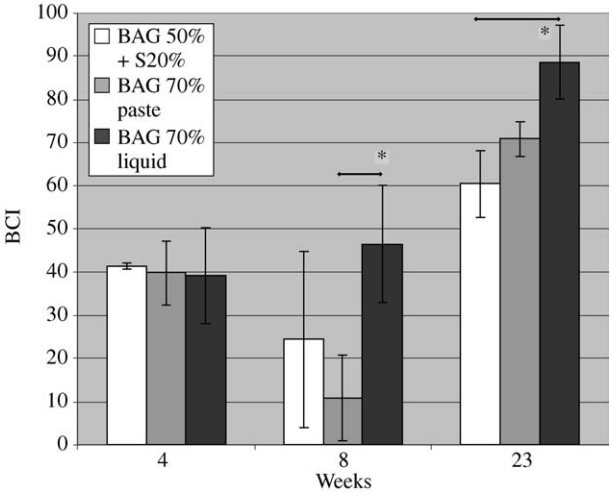

Figure 8 The BCI (average values and standard deviations) indicating bone ongrowth (%) on the composite surface. BCI was highest with BAG 70% liquid with significant difference ($* = p < 0.02$, Tukey's test) to paste form at eight weeks and to BAG 50% + S 20% at 23 weeks.

TABLE III The BBI (number of glass-bone contacts/cm), the BCI (%), and bone ingrowth (incidence and area %) in cancellous bone defects filled with different injectable BAG/P(CL/DL-LA) composites

	BAG 70% liquid	BAG 70% paste	BAG 50% + S 20% liquid
Bone bioactivity index (SD)	24.1 (1.9)	9.1 (2.2)	10.1 (4.7)
Bone coverage index (SD)	88.6 (8.4)	70.9 (4.1)	60.5 (7.7)
Bone ingrowth			
Incidence %	61	82	60
Area % (range)	6 (0.6–16.2)	8 (0.9–26.4)	6 (2.1–14.1)

Average values and standard deviations (SD) at 23 weeks (* = $p < 0.02$, Tukey's test).

eight weeks with BAG 70% paste was significantly lower than with BAG 70% liquid. Both glass granule size ranges showed equal bone coverage. Copolymer without bioactive glass showed no direct bone ongrowth (Fig. 9(a)).

The high rate of bone ongrowth indicates good osteoconductivity. In the literature, the numerical histometric values of the contact area between the injectable material and bone have been varying depending on experimental and methodological variations. For example, composite cements of hydroxyapatite/BIS-GMA, BIS-MEPP, TEG DMA resins (50% of bone contact at eight weeks in rabbits, [5]), and bioactive glass–ceramic/Bis–GMA based resins (up to 57.8–78.7% of bone in 4–26 weeks in rats [6]) have been tested. It is apparent that in the present study the high amount of bioactive glass granules of the composite, 70 wt %, and the relatively long observation time, 23 weeks, have an important influence on the amount of bone ongrowth. In another study, similar composite with 60 wt % of BAG yielded lower values of direct bone contact, 45 ± 30% in trabecular bone at 16 weeks [12]. In our study, the proportion of bone at the interface continuously increased, whereas that of connective tissue decreased during the observation period indicating the contact area to turn into bone.

Cortical bone repair as lamellar bone formation occurred in 12% of the medial condylar bone defects filled with composites at eight and 23 weeks (Figs. 9 and 10). Most often the implant surface was covered with tight collagenous tissue.

Bone ingrowth

The ingrowth of new bone into the implants was seen often, the incidence being higher in the BAG 70% paste group than in other groups (Fig. 10(b), Table III), however, without significant difference. The addition of sucrose in the composite BAG 50% + S 20% did not improve the performance, but tendency to thin tunnel formation with tissue ingrowth into the implant was observed (Fig. 10(c)). The implants with smaller glass granules did not show bone ingrowth at all. This may be due to insufficient space around the glass granules for the cellular invasion and bone growth. The percentage of bone ingrowth into the composites varied between 6 and 8% at 23 weeks (Table III) calculated from the percentage of trabeculae in the intact rabbit femoral trabecular bone. Slow degradation rate of the copolymer is apparently the influencing factor that retarded the ingrowth process. With other injectable materials the ingrowth rate has been variable. Welch *et al.* [20] used resorbable tetracalcium–dicalcium phosphate cement (Bone Source®) in goats and observed new bone volume fraction increase from 16% at six weeks to 44% at two years. Frankenburg *et al.* [21] observed 23–26.2% new bone ingrowth in 78 weeks using carbonate apatite cement (Norian® SRS®) in dogs, Gauthier *et al.* [8] 30.8% in 12 weeks using biphasic calcium phosphate with cellulosic polymer in rabbits, and Dupraz *et al.* [7] reported relative percentage of bone to be 34% at 26–78 weeks using HA-TCP-cellulose composite in rabbits.

The bone ingrowth rate in our study was expected to be better, since *in vitro* water absorption and copolymer degradation rate of the same composite materials suggested a faster resorption *in vivo*, especially with granule size $< 45\,\mu m$ [15]. Increased resorption and degradation would create porosity leading to tunnelling network necessary for bone ingrowth. However, the ingrowth rate was only moderate. It is apparent that porosity like tunnel formation, observed by sucrose addition in our study, is possible to be created. In the literature there are reports on techniques to increase the porosity with porogenic agents such as sodium chloride or ammonium bicarbonate [9, 22]. However, the porosity of the injectable carrier may no more exist *in vivo* if collapse of the structure occurs during injection or moulding of the material. Testing of BAG/polymer composites *in vitro* does not seem to adequately predict their behaviour *in vivo*, because the physiological conditions in living bone are different, and in this respect less favourable, regarding the fluid perfusion compared to incubation in water or in simulated body fluid.

In an earlier study using non-degradable liquid acrylate, multinucleated cells were observed, and the hardened acrylic acid polymer had a blocking effect that prevented bone healing [23]. By using the present composites, Glepron, foreign body cellular reaction was very low, and resorbing osteoclasts [22], or numerous mononuclear macrophages and giant multinucleated cells due to resorption [7, 8], or sarcomatous cell changes as reported by Nakamura *et al.* [24] were not observed. The absence of bone growth using the ε-caprolactone-lactide copolymer without bioactive glass corresponds to the results of Ekholm *et al.* [25].

The often demanded property of high compression strength for bone substitutes (Fig. 1) is not mandatory in cancellous, trabecular bone. The compressive strength of

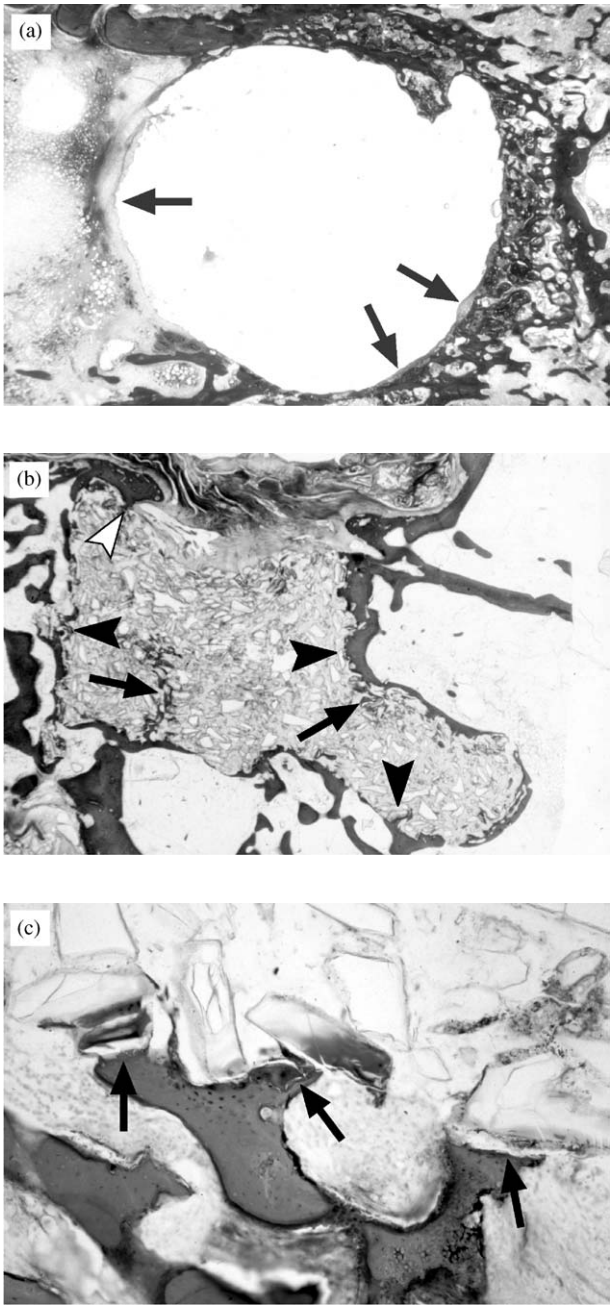

Figure 9 Histological images. (a) Control defect in cancellous bone filled with P(CL/DL-LA) without bioactive glass at four weeks. Connective tissue layer lines the copolymer implant (arrows). Masson–Goldner stain, original magnification × 10. (b) Defect filled with BAG 70% liquid at eight weeks. Contact between glass granules and bone (black arrow heads), ingrowth of new bone (arrows), and cortical lamellar bone repair (white arrow head). Masson–Goldner stain, original magnification × 10. (c) BAG 70% liquid at 23 weeks. Detail of the bonding phenomenon indicated by direct bone apposition on the bioactive glass granules (arrows). Masson–Goldner stain, original magnification × 30.

the present composites corresponds to the values of human cancellous bone. Mechanical properties of some calcium phosphate cements, for example, Norian® SRS® [4, 21] have initial compression strength corresponding to that of trabecular bone, and they reach their final strength, exceeding values for trabecular bone, in 24 h. Norian® SRS® has been used in the treatment of trabecular bone fractures of the distal radius and in tibial plateau fractures [26, 27]. Clinically, high initial strength is not always necessary in trabecular bone, because when used in small cavitary bone defects the filling material will not always meet the loading forces.

Regeneration of cartilage

Cartilage formation at the level of articular surface was evaluated from the 25 defects drilled through the intercondylar cartilage. Signs of cartilage regeneration were observed equally at all observation times. New cartilage tissue covering the implant was observed in 64% (16/25) of the defects filled with composites, however, even in 82% of the defects filled with BAG 70% liquid. Tissue was characterised by chondroblasts and both mature and immature chondrocytes, and increased intercellular matrix stained slightly metachromatically with toluidin blue and safranin. Also, small calcified bone islets and fat cells were seen (Figs. 11 and 12). Intensive staining of the surface reaction layer of the glass granules was characteristic below the new cartilage tissue, particularly with the Masson–Goldner trichrome stain. In the histological scoring of cartilage (Table II) this neocartilage scored 10.1 points on average (range 4–16) versus 7.3 (range 6–8) in the unfilled control defects.

In recent years the interest to use biomaterials for cartilage repair has yielded some progress. Tissue engineering concepts, commonly transplanted chondrocytes seeded in polymeric devices made from polylactic acid (PLA), polyglycolic acid (PGA) or porous collagen/Dacron® [18, 28–31] have induced cartilage formation in experimental studies. It is worth mentioning that cartilage formation has also been evident without transplanted cells in combination with a PGA device [18]. Formation of undifferentiated mesenchymal tissue on PLA in cartilagineous and subchondral bone defects has been observed [32]. Taking this into account, the cartilage formation in our study was encouraging and may be the pioneer observation of cartilage tissue repair on the injectable aliphatic polyesters without any support of simultaneous chondrocyte or cartilage transplantation. However, there are observations on bioactive glass supported cartilage differentiation related to biomaterials. Hyaline cartilage repair has been reported on the surface of solid bioactive glass cones [33] and on porous bioactive glass cones [34]. Thus, it seems likely that the influence of bioactive glass in the present composite is an important stimulating factor for the regeneration of cartilage tissue observed in this study.

Degradation of the composites

The signs of degradation were relatively low. Higher translucency in sections with Masson–Goldner trichrome stain was seen at 23 weeks at the outer parts of the composite. Some replacement of the composite by tissue was observed as bone ingrowth. Resorption occurred mostly at the interface as the amount of glass granules in contact with tissue increased over the observation period. This was indicated by changes in the BBI (Fig. 6).

Bone repair of the unfilled defects

Trabecular cancellous bone repair of the unfilled control defects $(N = 7)$ was incomplete still at 23 weeks

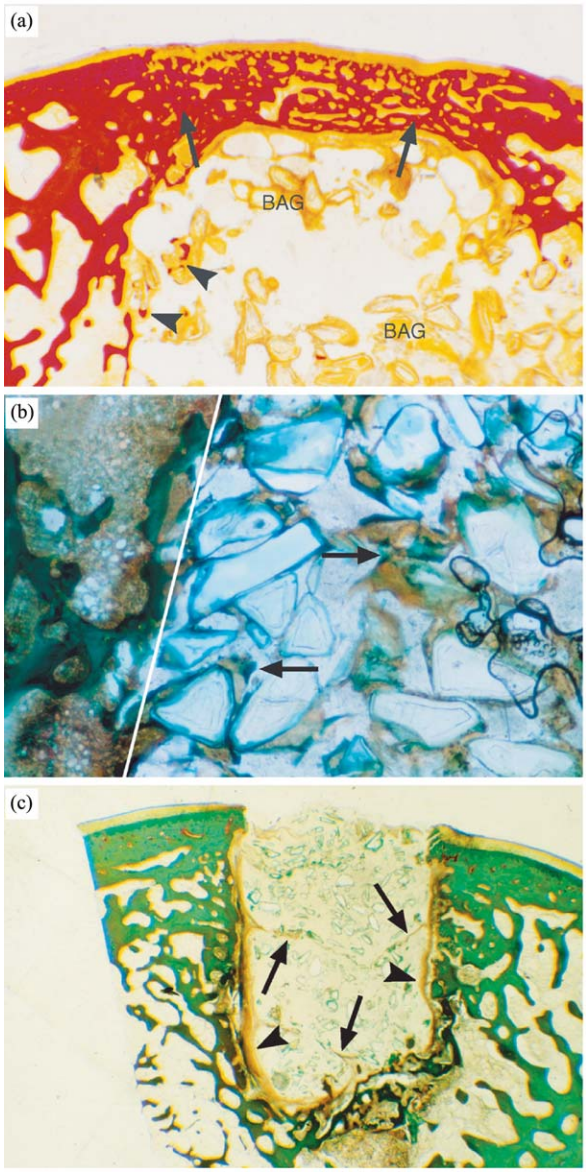

Figure 10 Histological images. (a) Cortical lamellar bone repair (arrows) covering a defect filled with BAG 70% paste at eight weeks. Arrow heads indicate bone ingrowth around the glass granules. BAG = bioactive glass. Van Gieson stain, original magnification × 12. (b) BAG 70% paste at four weeks. Ingrowth of new bone (arrows) into the composite between and along the surfaces of BAG granules. White line indicates the interface between the composite and the host bone. Masson–Goldner stain, original magnification × 35. (c) BAG 50% + S 20% at eight weeks. Addition of sucrose has resulted in thin tunnelling porosity and loose connective tissue ingrowth (arrows). Mainly connective tissue (arrow heads) lines the composite. Masson–Goldner stain, original magnification × 10.

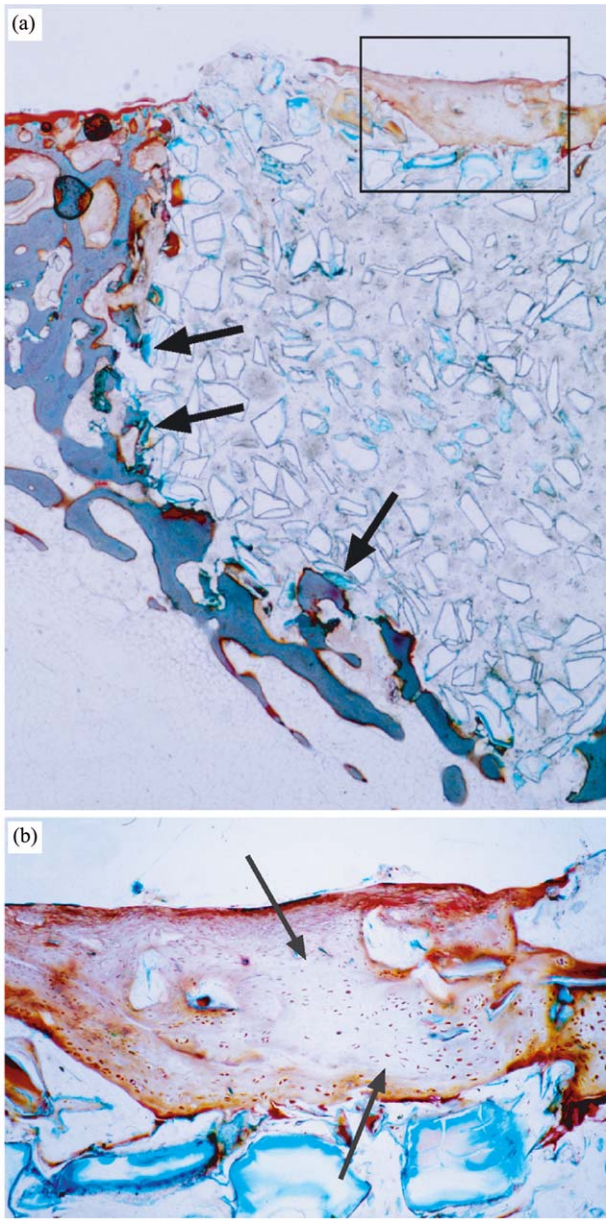

Figure 11 Histological images. (a) Cartilage tissue (rectangle) on the upper surface of BAG 70% liquid at eight weeks in cavitary defect drilled through the intercondylar cartilage. Some glass–bone contacts are indicated by arrows. Note the intensive staining of the surface reaction layer on the glass granules below the cartilage. Masson–Goldner stain, original magnification × 10. (b) Detail (rectangle in Fig. 11(a)) illustrating cartilage cells at different stages of maturation (arrows). Original magnification × 40.

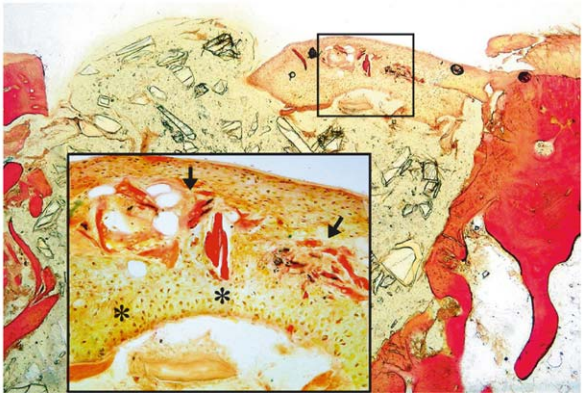

Figure 12 Cartilage formation (small rectangle) on BAG 70% liquid covering the composite at 23 weeks in cartilagineous subchondral bone defect. Van Gieson stain, original magnification × 12. Detail (large rectangle) illustrates small islets of bone (arrows) inside the cartilage. Mature chondrocytes (*) close to composite surface.

illustrated by decreased trabecular bone area consisting of 7.6% (3.3–11.2%) of bony trabeculae in the defect. No cortical lamellar bone repair occurred during the observation period. The normal intact trabecular bone structure in the distal femur of rabbits consisted of 31.6% (23.7–38.1%) of trabeculae.

4. Conclusions

We conclude that the basic concept to combine the copolymer P(CL/DL-LA) and particulate BAG fulfils several of the properties necessary for ideal injectable bone filler. The thermoplastic composites, Glepron, were bioactive and biocompatible, osteoconductive, ductile and conveniently injectable with short setting time. Further engineering is needed to accelerate and adjust resorption, and to increase the porosity of the carrier matrix in order to increase bone ingrowth rate. Cartilage formation and differentiation of cartilage cells on the glass/copolymer composite surface was a new and interesting observation suggesting potentials to develop technology for cartilage reconstruction possibly without cooperation of transplanted chondrocytes.

Acknowledgment

The authors thank the National Technology Agency of Finland, TEKES, and Academy of Finland (Centre of Excellence, Bio and Nanopolymers Research Group) for financial support.

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Received 25 June 2003
and accepted 21 January 2004