J Mater Sci: Mater Med (2011) 22:1111–1118
DOI 10.1007/s10856-011-4290-y

Mechanical strength of ceramic scaffolds reinforced with biopolymers is comparable to that of human bone

S. S. Henriksen · M. Ding · M. Vinther Juhl · N. Theilgaard · S. Overgaard

Received: 24 November 2010 / Accepted: 10 March 2011 / Published online: 24 March 2011

Abstract Eight groups of calcium-phosphate scaffolds for bone implantation were prepared of which seven were reinforced with biopolymers, poly lactic acid (PLA) or hyaluronic acid in different concentrations in order to increase the mechanical strength, without significantly impairing the microarchitecture. Controls were un-reinforced calcium-phosphate scaffolds. Microarchitectural properties were quantified using micro-CT scanning. Mechanical properties were evaluated by destructive compression testing. Results showed that adding 10 or 15% PLA to the scaffold significantly increased the mechanical strength. The increase in mechanical strength was seen as a result of increased scaffold thickness and changes to plate-like structure. However, the porosity was significantly lowered as a consequence of adding 15% PLA, whereas adding 10% PLA had no significant effect on porosity. Hyaluronic acid had no significant effect on mechanical strength. The novel composite scaffold is comparable to that of human bone which may be suitable for transplantation in specific weight-bearing situations, such as long bone repair.

S. S. Henriksen (✉) · M. Ding · S. Overgaard
Orthopaedic Research Laboratory, Department of Orthopaedic Surgery and Traumatology, Odense University Hospital, Institute of Clinical Research, University of Southern Denmark, Sdr. Boulevard 29, 5000 Odense C, Denmark
e-mail: susan.henriksen@ouh.regionsyddanmark.dk

M. Vinther Juhl · N. Theilgaard
Centre for Plastics Technology/Medical Devices, Danish Technological Institute, Gregersensvej 7, 2630 Taastrup, Denmark

1 Introduction

A biocompatible and strong scaffold with osteoconductive and -inductive properties is highly needed for specific load bearing purposes in orthopaedic and maxillo-facial surgery. Today, allograft and autograft are the preferred graft materials used in the clinic for revision surgery of joint replacements and spine fusion, but an alternative material is needed, as allograft and autograft are associated with several disadvantages and in short supply [1]. The process of harvesting autograft is associated with an increased risk of donor site morbidity, as well as difficulties in harvesting enough viable bone material [2]. Allograft is associated with slower bone incorporation, because of possible immunological host reactions [3].

The natural calcium-phosphate compound hydroxyapatite (HA) has been used as a bone substitute for many years, because of its biocompatibility and osteoconductive properties. When implanted in bone, HA is slowly resorbed and replaced by new bone growth [4]. Combining HA with the more rapidly soluble beta-tricalcium-phosphate (β-TCP) in an optimal ratio, results in an osteoconductive scaffold with optimal calcium-phosphate donor potential, because of the different dissolution rates of the materials. Unfortunately, HA/β-TCP ceramic scaffolds for implantation in bone are rigid and fragile, and the mechanical properties are not comparable to human bone. Therefore, different attempts to reinforce and improve HA/β-TCP ceramic scaffolds have been tried. Biocompatible polymers such as poly lactic acid (PLA), polyglycolic acid and polycaprolactone have been added to ceramic scaffolds in order to increase the mechanical strength of the scaffolds without decreasing the porosity of the final composite [5, 6].

PLA is a biocompatible polymer consisting of long chains of lactic acid. PLA is degraded by hydrolysis to lactic acid, which in turn is excreted as water and carbondioxide via the tricarboxylic acid cycle. It is used as a suture material because of its strength, biocompatibility and solubility [7, 8].

Low molecular weight (LMw) hyaluronic acid (HyA) (approximately 30 kDa) has been shown to increase osteogenic differentiation of mouse calvarial mesenchymal stem cells in vitro [9]. Endogenous HyA plays a significant role in embryogenesis, with its involvement in migration, proliferation and differentiation of mesenchymal cells [10, 11]. Thus, HyA could make up a resilient composite substitute with osteoconductive as well as osteoinductive potential.

The aim of this study was to investigate the microarchitecture and mechanical properties of pure ceramic and composite scaffolds for bone implantation. We hypothesized that the addition of a resilient biopolymer to a ceramic scaffold would enhance the mechanical strength of the composite scaffold without impairing the microarchitecture and possible osteoinductivity of the material.

2 Materials and methods

2.1 Materials

Seven groups of different composite scaffolds and one group of pure ceramic (HA/β-TCP) were produced and prepared in cubes (10 × 10 × 10 mm) by The Danish Technological Institute (Taastrup, Denmark) as described below (Table 1).

Biphasic calcium-phosphate material consisting of HA and β-TCP with the stoichiometric composition 70/30 was obtained as a powder from FinCeramica (Faenza, Italy). Poly(D, L)-lactic acid, PDLLA (50% D-PLA, 50% L-PLA) Mw = 308000 Da, was provided by PHUSIS (Saint Ismier, France). Hyaluronic acid (HyA): Mw = 0.05 MDa low molecular weigth (LMw), and Mw = 1.0 MDa medium molecular weight (MMw) was provided by Novozymes A/S (Bagsvaerd, Denmark).

Table 1 Overview of the materials produced and analyzed in this study

Group	Composite material	*n*
1	No polymer, pure HA/β-TCP	6
2	5% wt PDLLA + HA/β-TCP	5
3	10% wt PDLLA + HA/β-TCP	6
4	15% wt PDLLA + HA/β-TCP	6
5	5% wt HyA-LMw + HA/β-TCP	5
6	5% wt HyA MMw + HA/β-TCP	6
7	5% wt PDLLA and 5% wt HyA-LMw + HA/β-TCP	6
8	5% wt PDLLA and 5% wt HyA-MMw + HA/β-TCP	6

2.1.1 *Preparation of the pure HA/β-TCP scaffolds*

The porous structures were prepared using a CO_2 foaming of a ceramic slip with a high polymer content, followed by binder removal and sintering at 1200°C [12].

2.1.2 *Method for deposition of polymers*

The polymers were introduced into the sintered porous HA/β-TCP ceramic scaffold by a solvent infiltration method (demineralised water for HyA and acetone for PDLLA). The polymer solution was introduced at room temperature; the scaffold was subsequently dried under vacuum and weighed. The process was repeated until the required polymer load was reached. For the composite scaffolds containing both PDLLA and HyA, the PDLLA was introduced first. The amounts of polymers added in this study are expressed as weight percent (wt%) of the total construct.

2.2 Evaluation of pore sizes

Pore sizes and interconnected pores were evaluated by microscopy.

2.3 Micro-CT scanning and 3D-reconstruction

All scaffolds were micro-CT (μCT) scanned using a high resolution microtomographic system (VivaCT 40, Scanco Medical AG. Brüttisellen, Switzerland) followed by 3D-reconstruction of images and 3D-microarchitectural analysis. The 3D-microarchitectural properties of the composite scaffolds were compared to those of the pure ceramic scaffold, HA/β-TCP. The mean porosities, scaffold wall thicknesses (ScTh), structural model indeces (SMI) and connectivity densities (CD) of the composite scaffolds were calculated. SMI characterizes the 3D structure types of the scaffold as a certain amount of rods and plates. The value of SMI lies between 0 and 3, when the structure consists of both rods and plates of equal thickness, depending of the volume ratio of rods and plates. An ideal plate-like structure will reflect a high mechanical strength and has a SMI value of 0, whereas an ideal rod-like structure will reflect a low mechanical strength and has a SMI value of 3 [13, 14]. CD is topological measurement used to describe the porosity and number of interconnected scaffold trabeculae of the material [15].

2.4 Mechanical testing

Following μCT scanning, destructive compression test of the scaffolds was performed on an 858 Bionix MTS hydraulic material testing machine (MTS Systems Corporation, Minneapolis, Minnesota). All materials were tested the same day, under the same dry conditions at room temperature. A load cell of 1 kN was used, and a static strain-gauge extensometer (Model 632.11F-20; MTS Systems Corporation) was attached to the testing columns close to the specimen. Load-deformation data were collected and converted to stress–strain data. Max load, ultimate stress (strength), Young's modulus (stiffness), and failure energy were calculated.

2.5 Statistics

Data are presented as mean ± SD. All data were assessed by One-way ANOVA, and the post hoc multiple comparisons were adjusted using Bonferoni's test or paired Student's *t*-test as appropriate. Value of $P < 0.05$ was considered statistically significant.

3 Results

3.1 Microarchitectural properties

Microscopic evaluation shows highly interconnected samples with the majority of pores between 300 and 700 µm and interconnections from 100 to 200 µm (Fig. 1).

Porosities of the composite scaffolds were affected by adding polymers i.e. polymers added to the scaffold decreased the porosity (Table 2). The porosities in groups (2, 4, 5) containing PDLLA or HyA-LMw had porosities of 67, 54 and 69% respectively. These porosities are significantly lower than the 79% of group 1. The porosities of all other groups (3, 6–8) were not significantly different from that of group 1.

SMI in groups 2, 4, 5 was significantly smaller ($P < 0.05$) than SMI in group 1. Group 2 had an overall mixed shape of structure type with more plates than rods. The SMI value seen in group 4 reflected a structure type consisting of plates. The negative value reflects the concave curvature caused by fenestrations in a flat plate. Group 5 had an SMI value reflecting a plate-like structure. Group 1, with no polymer added, had an overall rod-like structure type. Composite scaffolds with PDLLA added (groups 2, 4, 5) have significantly different structure types as compared to group 1, whereas the structure type of group 3 were not significantly different. Groups 6–8 had an overall mixed structure type, not significantly different from that of group 1.

Scaffold thickness (ScTh) was also evaluated. The thicknesses of all composite scaffolds (groups 2–8) were greater than that of group 1, but only the thicknesses of groups 3–5 were significantly different from group 1. The thickness of group 4 was even significantly greater ($P < 0.05$) than that for all other groups, except for that of group 5. There were no significant differences in connectivity density between groups.

The changes in microarchitecture (porosity, ScTh) of the composite ceramics are visualised in Fig. 2.

3.2 Mechanical properties

Results from destructive compression test can be seen in Fig. 3. The strength of the composite scaffolds increased significantly with PDLLA infiltration. Figure 3a revealed a dose–response effect with the addition of PDLLA from 5–15% seen in the groups 2–4. Group 4 showed significantly higher max load and ultimate stress when compared to group 1 and all other groups. Group 3 exhibited a significantly higher strength than all other groups, except group 4. Groups 2 and 7 were also significantly different from group 1 regarding the max load applied on the materials. HyA LMw (group 5) had a significant effect on overall strength, when compared to group 1.

Young's moduli for all composite scaffolds were affected by the addition of polymers (Fig. 3c). Group 4 had a significantly higher stiffness than all other groups. The stiffness of group 4 was approximately 10-fold higher than that of group 1, whereas the stiffness seen in group 3 was approximately 6-fold higher than that of group 1. The stiffness for group 3 was significantly higher than that of groups 1 and 2.

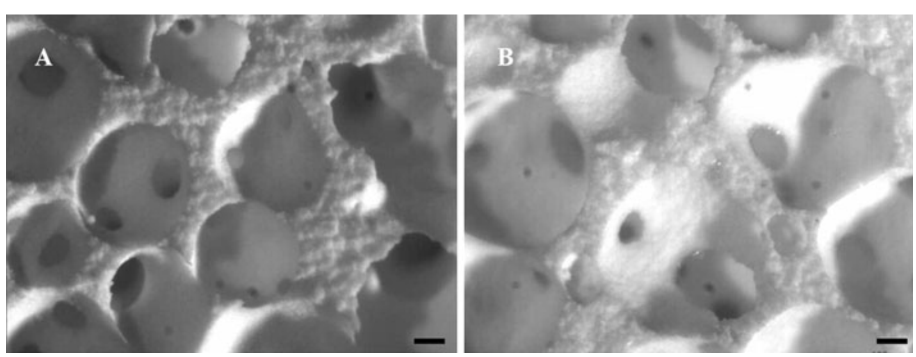

Fig. 1 Microscopic evaluation of pores and interconnecting pores of two selected scaffolds. Microscopic evaluation shows highly interconnected samples with the majority of pores between 300 and 700 µm and interconnections from 100 to 200 µm. Scale bar represents 100 µm. Group 1 (HA/β-TCP) (**a**). Group 4 (HA/β-TCP + 15% PDLLA) (**b**)

Table 2 Results from μ-CT analysis and evaluation of the materials in this study

Groups	Porosity (%)		SMI (−)		ScTh (mm)			CD (mm^{-3})	
	Mean ± SD	*P*-values comparisons with group 1	Mean ± SD	*P*-values comparisons with group 1	Mean ± SD	*P*-values comparisons with group 1	*P*-values comparisons with group 1	Mean ± SD	*P*-values comparisons with group 1
1	79 ± 3	–	2.31 ± 0.29	–	0.15 ± 0.01	–	<0.001	12.4 ± 4.3	–
2	67 ± 2	0.03	0.71 ± 0.26	0.04	0.19 ± 0.00	0.06	<0.05	13.4 ± 1.6	0.916
3	71 ± 7	0.10	1.15 ± 0.95	0.11	0.20 ± 0.03	<0.05	<0.05	11.1 ± 3.4	
4	54 ± 13	<0.001	1.46 ± 2.61	<0.001	0.25 ± 0.07	<0.001	–	11.2 ± 5.1	
5	69 ± 5	<0.05	0.36 ± 0.91	<0.05	0.22 ± 0.04	<0.05	0.14	10.4 ± 5.0	
6	71 ± 7	0.10	1.24 ± 0.95	0.14	0.19 ± 0.03	0.08	<0.05	11.3 ± 2.6	
7	72 ± 10	0.16	1.29 ± 1.12	0.16	0.18 ± 0.00	0.14	<0.05	10.6 ± 4.7	
8	73 ± 10	0.22	1.37 ± 1.09	0.20	0.18 ± 0.03	0.16	<0.05	10.3 ± 3.9	

P-values less than 0.05 were considered significant (ANOVA)

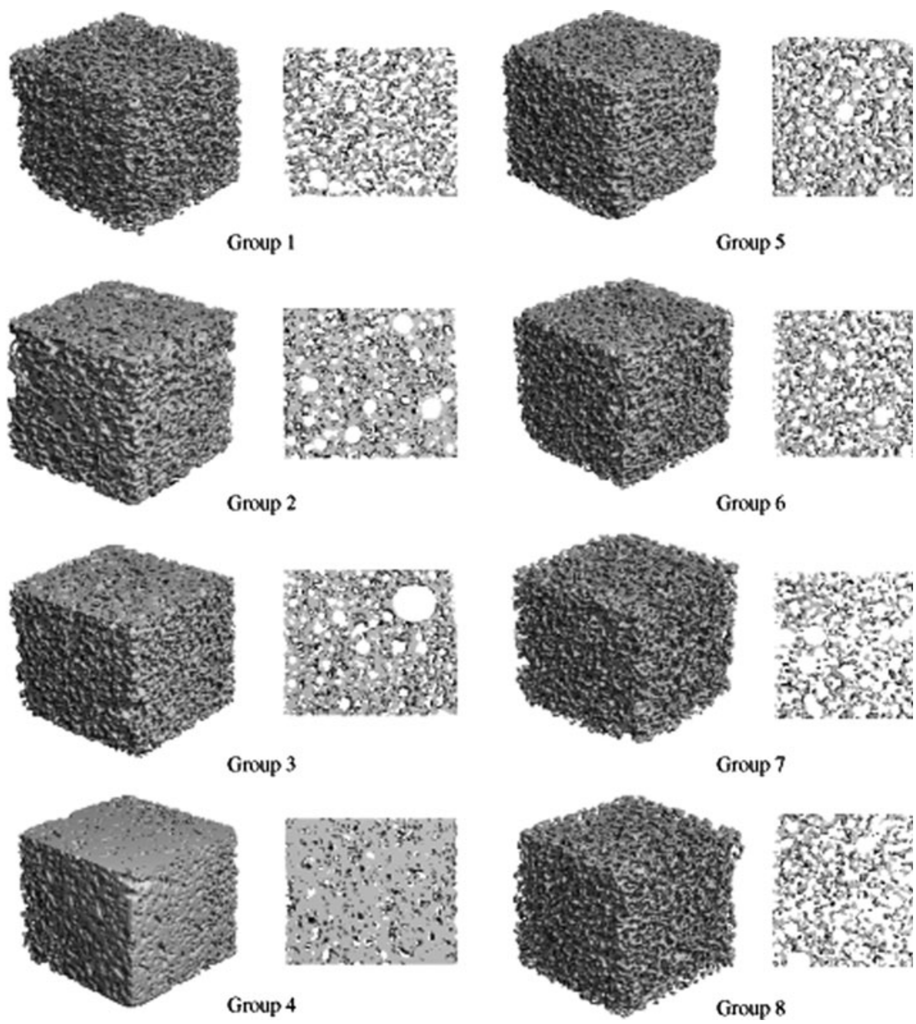

Fig. 2 Three-dimensional reconstructions from μ-CT images of scaffold materials prepared in cubes (10 × 10 × 10 mm). Right: Reconstructions of cubes. Left: cross sections of cubes. Microarchitecture is visibly altered by adding polymers to the ceramics. This is especially evident for groups 2–4, where PDLLA is added

Failure energy was also affected by the addition of PDLLA. Failure energy was significantly increased and more than 4-fold higher for group 4 than compared to all other groups.

4 Discussion

This study investigated the microarchitecture and mechanical properties of composite scaffolds reinforced

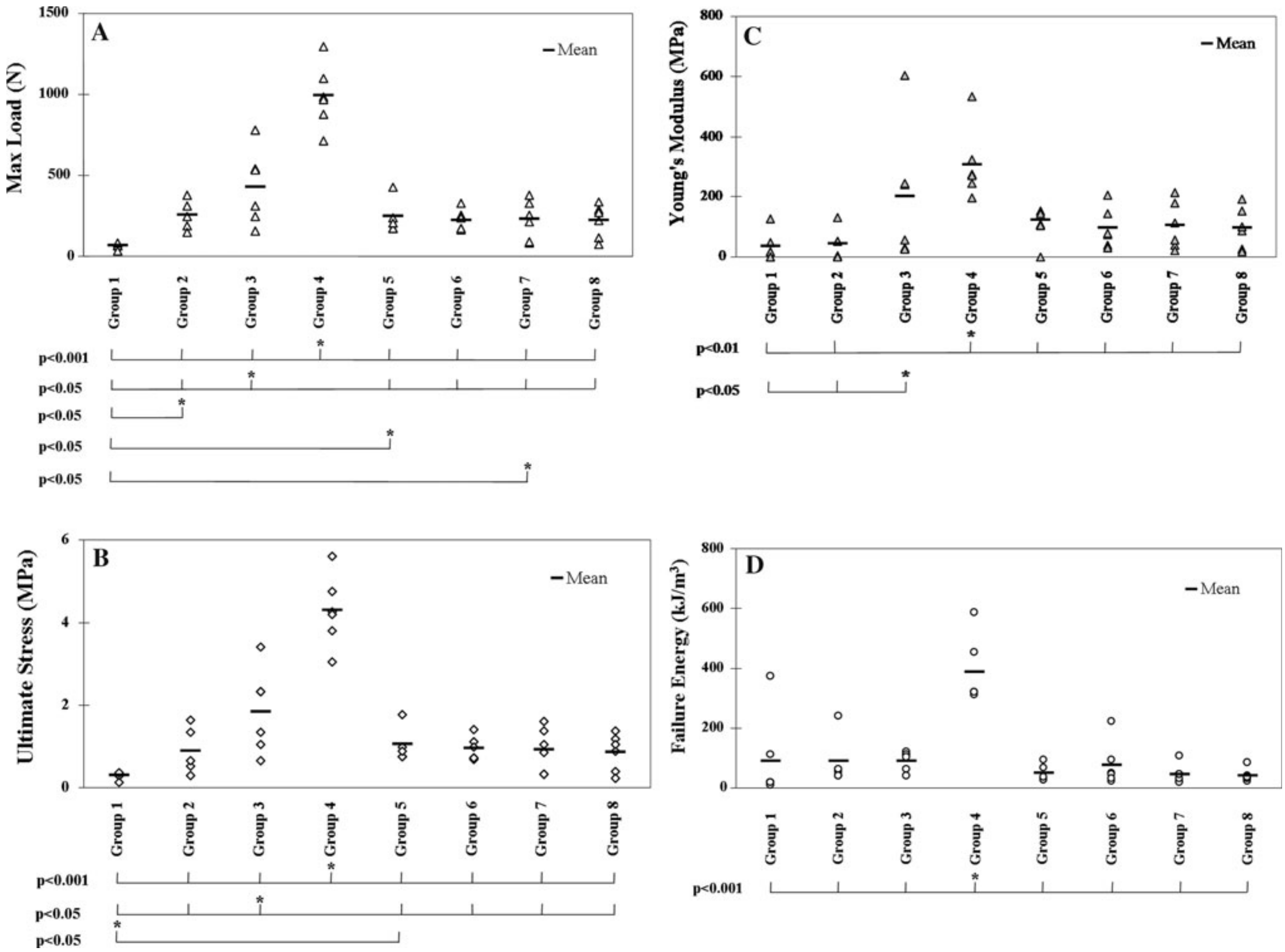

Fig. 3 Exact description of materials can be viewed in Table 1. Destructive compression test show the effects of mechanical properties of polymers added to the ceramic scaffolds. All data points are presented. Mean is indicated by horizontal bar. Significant differences between groups are indicated by *asterisks*. *P*-values less than 0.05 were considered significant (ANOVA). Max load (N) (**a**), Ultimate stress (MPa) (**b**), Young's modulus (MPa) (**c**), Failure energy (kJ/m^3) (**d**)

with resilient biopolymers (PDLLA and HyA). We demonstrated that adding a resilient biopolymer (10% PDLLA) to the calcium–phosphate scaffold materials does not impair the microarchitecture with regards to porosity, SMI, ScTh and CD. Furthermore, the addition of a polymer to the scaffold enhanced the mechanical strength of the scaffold making it comparable to that of human cancellous bone [16] and hence useful for implantation in bone in specific weight-bearing areas.

The microarchitecture of a scaffold can typically be characterized by its porosity, interconnected pore size and scaffold size and shape. Porosity is important for cell migration and proliferation, transfer of nutrients and vascular ingrowth. Sufficient mechanical strength of a scaffold is important for ensuring mechanical stability for early anchorage of the scaffold to the surrounding bone, and ultimately for optimal bone formation [17]. However, mechanical strength is influenced by the microstructure as low porosity (<70%) is correlated with a high mechanical strength [17]. Consequently, when designing a resilient composite scaffold material for bone implantation, the risk of lowering the porosity of the scaffold material when adding polymers must be considered.

Thickness of the trabeculae of the scaffold (ScTh) is also an important characteristic and is related directly to the mechanical strength of the material. Structure type (reflected by SMI) has been shown to correlate well with the ultimate strength of a material resembling human trabecular bone [18, 19].

The porosities seen in the composite materials described in this study range from 54 to 73%. The porosities are lowered as a consequence of adding polymers. The porosity of the pure ceramic in group 1 is high; consequently with low mechanical strength, stiffness and failure energy when compared to the PDLLA infiltrated scaffolds (groups 3, 4). Overall, adding resilient biopolymers to the ceramic improved the mechanical strength significantly; partially explained by a reduction of the porosity. Nevertheless, for bone formation porosity must be large enough to allow cell migration and proliferation within the scaffold, as well as supply and allow for exchange of oxygen and nutrients through ingrowth of capillaries. A pore size of >300 μm has been shown to encourage osteogenesis whereas a pore size of ~100 μm is generally considered to be the minimally accepted pore size for induction of new bone ingrowth [20]. The interconnected pores and pore sizes (300–700 μm) found in the materials analyzed in this study are all within the limits of what is needed for optimal osteoinduction.

Studies have shown a correlation between high porosity and increased bone ingrowth, but as a result, the mechanical stability of the scaffold is reduced [21]. Thus, mechanical strength and stiffness of the scaffold must be increased without compromising the porosity of the material, and ultimately affecting stimulation and growth of new bone. The addition of HyA (groups 5 and 6) results in a decreased porosity compared to the pure ceramic in group. The porosities are, however, still optimal for facilitation of bone ingrowth. The mechanical strength seen in these two groups are not significantly better than that of pure ceramic, although approximately 3-fold higher. The relatively low strength and stiffness could be explained by the fairly low amount of HyA LMw/MMw added to the ceramics (5%). Adding 10 or 15% HyA LMw or MMw to a ceramic material might also reveal a dose–response effect regarding the strength parameters as seen with groups 2, 3 and 4. The structure of the two composite scaffolds with LMw and MMw HyA are overall thicker, plate-like or a mix of rods and plates, respectively. The combination of 5% HyA LMw or MMw with 5% PDLLA as seen in groups 7 and 8, show similar characteristics as groups 5 and 6 regarding porosity and mechanical strength. The porosities are acceptable for a composite scaffold, but the mechanical strengths, stiffnesses and failure energy are low. Probably due to the shape of the trabeculae, which are found to be generally mixed with a tendency towards a more rod-like nature, but also because of the relatively low amount of polymer added (5%).

The addition of 15% PDLLA (group 4) significantly decreased the porosity of the material compared to the pure ceramic from ~80 to 54%. This level of porosity is considered to be too low for bone ingrowth [21]. The mechanical properties of this composite scaffold are, however, found to be the highest for all groups, reflected by the structural properties obtained from the μCT analysis. The SMI index reveals a highly plate-like structure although concave, and the ScTh is almost two-fold that of the pure ceramic. The SMI values also correlate well with the ultimate strength of the composite scaffolds with PDLLA added as previously described. Thus, increased mechanical strength is obtained at the expense of a critically lowered porosity [16]. Meanwhile, a porosity of 71%, as obtained with the addition of 10% PDLLA (group 3), will facilitate bone ingrowth. Mechanical properties of this composite scaffold also have a significantly higher mechanical strength than the pure ceramic in group 1. A dose–response effect can be seen in composite scaffolds with 5, 10 and 15% PDLLA added. The addition of 5% PDLLA has no significant effect on the strength of the material. The porosity is 67% which is also considered to be optimal for bone ingrowth. The scaffold materials with PDLLA added have an overall mixed structure, and have a significantly greater ScTh than that of the pure ceramic.

The elasticities (Young's moduli) observed for all the composite scaffolds are, however, relatively low when compared to healthy trabecular bone [16, 22, 23]. This is presumably due to the absence of cells and extracellular matrix components such as collagen. Especially collagen is considered a contributor to the ductility of bone [24, 25]. Other parameters such as structure type (SMI and CD) and thickness are highly comparable to that of human cancellous bone, which is also reflected by the mechanical strength of the scaffold material [18]. In this study we chose to combine PDLLA and HyA with a ceramic scaffold in order to create a strong and elastic composite scaffold. In an aqueous solution, HyA is shaped as a stiffened random coil with a highly hydrated volume [11]. This structure is highly correlated with the compressive resilience of the polymer. However, testing the compressive strength of the composite scaffolds was done using dry samples, and this might have had an influence on the stiffness of the composite scaffolds with HyA. The high resilience associated with the hydrophobic PDLLA is not due to its ability to bind water, but more to its bonds and overall structure [7]. Once in contact with water, the degradation of PDLLA will begin; thus lowering the strength of the composite scaffold. The expected degradation of the composite scaffold is, however, slower than that seen for the pure polymer, and should not affect the mechanical property of the composite scaffold once implanted in bone [26]. However, the degradation could create an acidic environment, which could be cell toxic if contained in a static environment e.g. in an in vitro cell culture. However, PDLLA used for these materials have been used for more than 20 years with proven excellent biocompatibility and absence of systemic or local toxicity. Therefore, these composite scaffolds reinforced with PDLLA are considered safe, even for implanting scaffolds of considerable sizes.

CD is, for all the materials in this study, found to be almost twice that of human cancellous bone [27]. This might be an effect of the foaming procedure used for preparation of the scaffold materials. Natural bone

Table 3 Overview of the properties of human cancellous bone found in the literature

Mechanical properties		Microarchitectural properties	
Parameter	Mean ± SD	Parameter	Mean ± SD
Young's modulus (MPa)	635 ± 386	SMI (−)	0.99 ± 0.52
Ultimate stress (MPa)	8.82 ± 3.50	Tb·Th (mm)	0.17 ± 0.32
Failure energy (kJ/m^3)	110 ± 52.0	CD (mm^{-3})	3.02 ± 0.80
Ding et al. [16]		Ding et al. [28]	
		Fields et al. [19]	

remodelling ensures a highly organized arrangement of trabeculae (Wolff's Law), whereas the foaming method produces material with a more random structure.

When comparing the materials in this study with human cancellous bone, some similarities are present (Table 3). The porosities of the materials in all groups, except for group 4 are comparable to cancellous bone. Trabecular thickness of cancellous bone is 186 ± 29 μm, as is the corresponding ScTh found in groups 2, 3, 6, 7 and 8 [28]. However, the mechanical strengths of the materials are only comparable to human cancellous bone when a relatively high dose of PDLLA is added (10–15%).

In conclusion, compared to human cancellous bone, the composite material reinforced with 10% PDLLA shows similar characteristics regarding mechanical strength and microarchitecture. Especially with regards to porosity, ScTh, SMI and ultimate strength, the material demonstrates a promising composite scaffold material for bone formation.

This scaffold might be useful for bone implantation for specific weight bearing areas. The addition of HyA had no significant effect on the mechanical properties tested in this study, but could still be a relevant component of a composite scaffold, because of its osteoinductive potential. Increasing the amount of HyA to the scaffold (e.g. 15%) might possibly improve the mechanical properties of the composite scaffold.

Acknowledgments This work was supported by The Danish Agency for Science, Technology and Innovation through the project "3D-Scaffolds". Project reference: 07-002634—3D-Scaffolds—Biomimetic 3D—structures for tissue regeneration.

References

1. Hing KA. Bone repair in the twenty-first century: biology, chemistry or engineering? Philos Transact A Math Phys Eng Sci. 2004;362(1825):2821–50.
2. Heary RF, Schlenk RP, Sacchieri TA, Barone D, Brotea C. Persistent iliac crest donor site pain: independent outcome assessment. Neurosurgery. 2002;50(3):510–6.
3. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. Clin Orthop Relat Res 2000 371:10–27.
4. Overgaard S, Soballe K, Josephsen K, Hansen ES, Bunger C. Role of different loading conditions on resorption of hydroxyapatite coating evaluated by histomorphometric and stereological methods. J Orthop Res. 1996;14(6):888–94.
5. Kim SS, Sun PM, Jeon O, Yong CC, Kim BS. Poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. Biomaterials. 2006;27(8):1399–409.
6. Eshragi S, Das S. Mechanical and microstructural properties of polycaprolactone scaffolds with one-dimensional, two-dimensional and three-dimensional orthogonally oriented porous architectures produced by selective laser sintering. Acta Biomaterialia 6(7):2467–2476.
7. Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. Eur Cell Mater. 2003;5:1–16.
8. Gunatillake P, Mayadunne R, Adhikari R. Recent developments in biodegradable synthetic polymers. Biotechnol Annu Rev. 2006;12:301–47.
9. Zou L, Zou X, Chen L, Li H, Mygind T, Kassem M, et al. Effect of hyaluronan on osteogenic differentiation of porcine bone marrow stromal cells in vitro. J Orthop Res. 2008;26(5):713–20.
10. Pilloni A, Bernard GW. The effect of hyaluronan on mouse intramembranous osteogenesis in vitro. Cell Tissue Res. 1998;294(2):323–33.
11. Bastow ER, Byers S, Golub SB, Clarkin CE, Pitsillides AA, Fosang AJ. Hyaluronan synthesis and degradation in cartilage and bone. Cell Mol Life Sci. 2008;65(3):395–413.
12. Habibovic P, Kruyt MC, Juhl MV, Clyens S, Martinetti R, Dolcini L, et al. Comparative in vivo study of six hydroxyapatite-based bone graft substitutes. J Orthop Res. 2008;26(10):1363–70.
13. Hildebrand T, Ruegsegger P. Quantification of bone microarchitecture with the structure model index. Comp Methods Biomech Biomed Eng. 1997;1(1):15–23.
14. Ding M, Odgaard A, Danielsen CC, Hvid I. Mutual associations among microstructural, physical and mechanical properties of human cancellous bone. J Bone Joint Surg Br. 2002;84(6):900–7.
15. Odgaard A, Gundersen HJ. Quantification of connectivity in cancellous bone, with special emphasis on 3-D reconstructions. Bone. 1993;14(2):173–82.
16. Ding M, Dalstra M, Danielsen CC, Kabel J, Hvid I, Linde F. Age variations in the properties of human tibial trabecular bone. J Bone Joint Surg Br. 1997;79(6):995–1002.
17. Byrne DP, Lacroix D, Planell JA, Kelly DJ, Prendergast PJ. Simulation of tissue differentiation in a scaffold as a function of porosity, Young's modulus and dissolution rate: application of mechanobiological models in tissue engineering. Biomaterials. 2007;28(36):5544–54.
18. Fields AJ, Eswaran SK, Jekir MG, Keaveny TM. Role of trabecular microarchitecture in whole-vertebral body biomechanical behavior. J Bone Miner Res. 2009;24(9):1523–30.
19. Mittra E, Rubin C, Qin YX. Interrelationship of trabecular mechanical and microstructural properties in sheep trabecular bone. J Biomech. 2005;38(6):1229–37.
20. Hulbert SF, Young FA, Mathews RS, Klawitter JJ, Talbert CD, Stelling FH. Potential of ceramic materials as permanently implantable skeletal prostheses. J Biomed Mater Res. 1970;4(3):433–56.
21. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005;26(27):5474–91.
22. Dalstra M, Huiskes R, Odgaard A, van EL. Mechanical and textural properties of pelvic trabecular bone. J Biomech. 1993;26(4–5):523–35.
23. Linde F. Elastic and viscoelastic properties of trabecular bone by a compression testing approach. Dan Med Bull. 1994;41(2):119–38.

24. Burr DB. The contribution of the organic matrix to bone's material properties. Bone. 2002;31(1):8–11.
25. Viguet-Carrin S, Garnero P, Delmas PD. The role of collagen in bone strength. Osteoporos Int. 2006;17(3):319–36.
26. Ehrenfried LM, Farrar D, Cameron RE. Degradation properties of co-continuous calcium-phosphate-polyester composites. Biomacromolecules. 2009;10(7):1976–85.
27. Ding M, Odgaard A, Linde F, Hvid I. Age-related variations in the microstructure of human tibial cancellous bone. J Orthop Res. 2002;20(3):615–21.
28. Ding M, Hvid I. Quantification of age-related changes in the structure model type and trabecular thickness of human tibial cancellous bone. Bone. 2000;26(3):291–5.