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Journal of the European Ceramic Society 31 (2011) 523–529

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Influence of gelatin coatings on compressive strength of porous hydroxyapatite ceramics

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Received 20 August 2010; accepted 2 November 2010
Available online 8 December 2010

Abstract

Porous ceramics prepared by the foam replication technique have a high porosity and low mechanical stability. It has been reported that coating such porous ceramics with gelatin allows for an improved compressive strength. Little details regarding the influence of important gelatin parameters such as concentration, temperature and drying conditions as well as bloom number which is a measure of the gel rigidity, on this toughening effect are available. This paper investigates the influence of these parameters on compressive strength of gelatin coated porous hydroxyapatite ceramics. It was found that concentration in the gelatin sols has a marked impact whereas sol temperature, bloom number and differing conditions during subsequent drying have only little influence on the compressive strength of the coated ceramics.

Keywords: Films; Composites; Strength; Apatite; Biomedical application

1. Introduction

Preparation of highly porous ceramics with interconnected pores is of interest for applications like, e.g. filter preparation, catalyst support or tissue engineering. A technique to obtain open porous ceramics is the so-called foam replication method, sometimes also called replica technique. According to this procedure, a template, usually a polyurethane sponge, is immersed in a ceramic slurry.[1] After removing excessive ceramic slurry the polymer sponge carries a coating of unsintered ceramic material. Drying and subsequent heating up to appropriate temperatures results in decomposition of the supporting polymer and sintering of the remaining ceramic framework.[2] Ceramics with an open interconnected porosity ranging from approx. 40 to 95% can be prepared by this technique.[3] However, due to their high porosity these ceramics have comparatively low mechanical stability. Depending on the ceramic material the compressive strength can get as low as 0.16 MPa for hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) at a porosity of 87%.[4] Similarly low values were reported for other ceramic materials such as Al_2O_3 (0.3 MPa, porosity 87%[5]). As such weak ceramics need to be handled with great care, increasing their strength is of interest. One way for improving their strength is to apply additional ceramic coatings. This can result in an unfavorable reduction of the intentionally high porosity. Another method for increasing their mechanical strength without compromising the high porosity is the application of an appropriate polymer coating. Reports about the successful use of polycaprolactone (PCL),[6] poly(lactic-co-glycolic acid),[7] poly(D,L-lactic acid),[8] dilactic–polylactic acid,[9] polymethylmethacrylate[10] or gelatin[11] can be found.

Gelatin is an interesting candidate as it is (i) readily available, (ii) comparatively cheap, (iii) dispersable in water, (iv) nontoxic and (v) can easily be applied as thin coating. As gelatin is produced from very different sources of animal collagen such as fish or cattle[12] it may contain different amino acids[13] in varying combinations. Hence, it is not a homogenous substance with a defined stoichiometry. Its properties depend on the conditions during preparation.[14] Two main types of gelatin are distinguished, i.e. type A and type B. The former is prepared under acidic conditions while the latter is obtained using an alkaline based process.[13] Solid gelatin can easily be dispersed in water to form a gelatin sol, which can transform into rigid gelatin upon cooling. This temperature induced process is reversible.

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doi:10.1016/j.jeurceramsoc.2010.11.004

The strength of gelatin gels is measured in gram bloom (abbr. gbloom). The higher the bloom number, the more rigid a gelatin gel.[15] When gelatin is applied as a coating its mechanical properties depend on factors like temperature and thermal history,[16,17] water content,[18] source of gelatin[12] and bloom number.[19] Albeit this multitude of factors influences the properties of gelatin coatings, little systematic work has been published regarding the influence of different conditions during the coating process on the properties of the gelatin coated ceramics.

It is thus the aim of the present work to investigate in more detail the influence of the following parameters (i) type of gelatin, (ii) temperature of gelatin sol, (iii) temperature during subsequent heat treatment as well as (iv) concentration of gelatin sols, on the mechanical properties of the gelatin coated porous ceramics.

2. Experimental

2.1. Preparation of porous ceramics by foam replication

HA-powder (brand name Ostim, Aap Biomaterials, Germany) was calcined at 350 °C for 1 h in air to equilibrate the surface area of differing delivered batches to ~60 m^2/g. The resulting calcined HA-powder was mixed with (i) water, (ii) a dispersing agent (Dolapix CE64, Zschimmer & Schwarz, Germany), (iii) viscosity and green strength modifiers 2-hydroxyethyl-methacrylat (Merck, Germany) and ethylenglykol (Merck, Germany), as well as (iv) a sintering aid ($CaNa_4(PO_3)_6$).

Ballmilling this mixture for 6 min leads to the formation of a shear thinning ceramic slurry (HA-content 73 wt%) which was used to impregnate polyurethane sponges (Zervos) with a pore content of 43 pores per inch. After air-drying the ceramic coated sponges for 24 h at room temperature, the following heat treatment regime was applied: (i) 40 K/h to 300 °C, 1 h holding time at 300 °C, (ii) 100 K/h to 1100 °C, 3 h holding time at 1100 °C, and (iii) 100 K/h to 1250 °C, 3 h holding time at 1250 °C. The resulting porous HA-ceramics had – according to X-ray tomography (μCT-40, Scanco Medical AG) – a mean pore/strut size of 0.88/0.26 mm. After sintering the specimens had a cylindrical shape with a height of ~3.3 cm and a diameter of ~1.9 cm.

2.2. Gelatin sol preparation

Porcine gelatin granules ($0.5 \leq d \leq 1.2$ mm) type A (Fluka, Germany) were allowed to swell in water for 30 min before heating to a designated temperature. Four different target temperatures were used, i.e. 50, 60, 70 and 80 °C. The gelatin concentration of the resulting gelatin sols will be given in this work in g gelatin per g water, i.e. “g/g”.

2.3. Viscosity measurements

Viscosity of the gelatin sols was measured with a rheometer (RS300, Haake) with a cone–plate detector head. The shear rate was increased in a step wise manner until 2500 s^{-1}. Measurements were carried out in controlled rate (CR) mode. The size of the gap between cone and plate was set to 0.06 mm. According to the instrument supplier, measured viscosities have a precision of ±5%. A thermostat (Haake) was used to keep the temperature of the cone, the plate and the sample between them at a designated temperature of 50 ± 1 °C.

2.4. Coating of porous ceramics with gelatin sols

Before coating the open porous HA-ceramics with gelatin sols, the ceramics were immersed in water which was than removed by a centrifuge. The water-wetted specimens showed an improved wettability during the subsequent gelatin sol coating. After immersing the water-wetted samples in a temperature controlled gelatin sol, the gelatin infiltrated HA-ceramics were put again into the centrifuge – now to remove excessive gelatin sol.

In order to improve the impregnation the ceramics were put into a chamber with reduced pressure during the pre-wetting with water and during the subsequent gelatin coating. This led to an improved removal of air out of pores.

After the gelatin coating all samples were allowed to dry at room conditions for 3 days. In order to study the influence of further heat treatments the dried gelatin coated samples were heated to (i) 100 °C under vacuum or (ii) 100 °C under atmospheric pressure.

The porosity of the coated gelatin samples was determined by weighing the uncoated and gelatin coated samples. Together with the dimensions of the samples and an assumed density of 3.16 g/cm^3 for HA[20] and 1.35 g/cm^3[21] for gelatin, the remaining porosity of the coated ceramics was calculated.

2.5. Mechanical testing

Mechanical testing of the gelatin coated ceramics was carried out with a universal testing machine (Z005, Zwick/Roell, Germany) at a crosshead speed of 1 mm/min. Ten samples were analyzed for each coating composition. The samples did not observe a smooth surface but due to their very porous nature some struts were slightly (order of ~100 μm) out of plane, i.e. protruded from the sample surface. Hence, in order to equilibrate the contact to the clamps a piece of ~1 mm thick soft rubber was placed between the sample surface and the rams. A similar procedure was applied earlier by Dam et al.[5] These authors found that (i) complying rams lead to a reduction in experimental scatter and (ii) the value for measured maximum compressive strength of the ceramic sample was not affected by the use of compliant rams.[5] Before starting a measurement samples were preloaded with 20 N which lead to a substantial compression of the rubber discs. Nevertheless the following should be noted: strain values given in this work must only be considered as rough approximates, first because of the use of the rubber discs and second as merely the machine displacement was recorded. The strength values given in this work are based on the projected surface area of the sample surface, thus the surface area is not corrected for porosity.

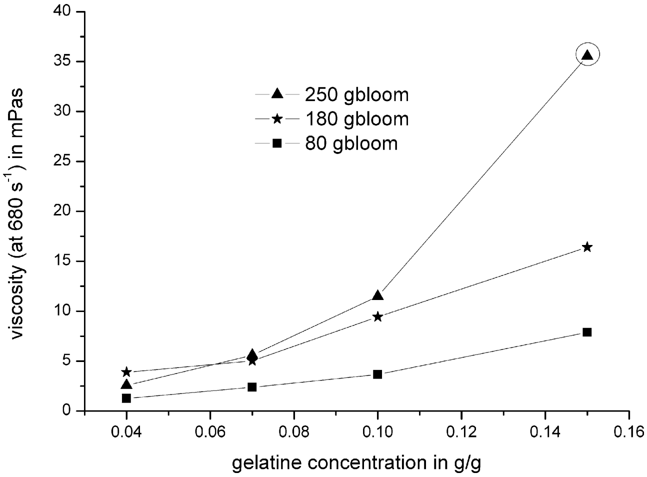

Fig. 1. Influence of concentration on viscosity for different types of gelatin (80, 180 and 250 gbloom, $T(\text{sol}) = 50\,°\text{C}$). The encircled point marks a sample with pronounced shear thinning. All other samples exhibited Newtonian behavior.

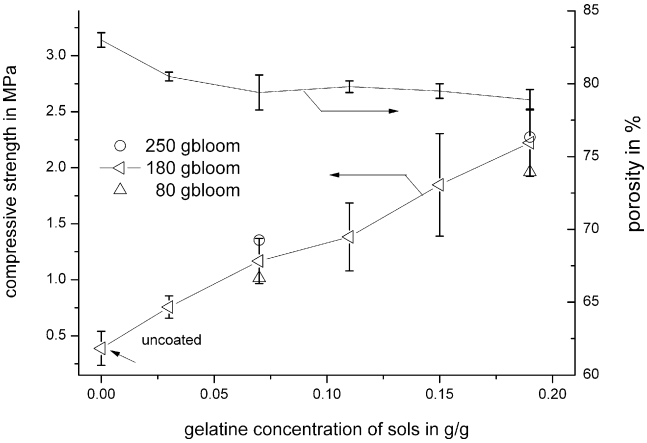

Fig. 2. Compressive strength of gelatin coated ($T(\text{sol}) = 50\,°\text{C}$) porous HA-ceramics as a function of gelatin concentration in the sols and type of gelatin.

2.6. Microscopy

Micrographs of the polyurethane sponge were taken with a light microscope (Keyence). Scanning electron microscopy (SEM) was used to study the fracture surfaces of the coated ceramics. Samples were fractured in their centre part. SEM micrographs were taken with a (SEM) Zeiss SUPRA$^{\text{TM}}$ 40 (Carl Zeiss SMT, Oberkochen, Germany) having a thermal field emitter. An accelerating voltage of 10 kV was applied. The specimens for SEM were previously coated with a very thin carbon layer in order to improve the electrical conductivity of the sample surface.

3. Results

At a temperature of 50 °C all prepared gelatin sols (80, 180 and 250 gbloom, $0.07 \leq c \leq 0.15$ g/g, $T(\text{sol}) = 50\,°\text{C}$) showed a rather Newtonian flow characteristic. In these sols no marked influence of shear rate on viscosity was found (details not shown). Similar Newtonian flow characteristics were reported by Leuenberger[22] who investigated the viscosity of gelatin sols (30, 140 and 300 gbloom at 0.1 g/g, $T(\text{sol}) = 40\,°\text{C}$). As can be seen in Fig. 1 where the viscosity at a shear rate of 680 s^{-1} is depicted, the following expected general trend was observed. The higher the gelatin concentration and the higher the bloom number the higher the viscosity. A nonlinear relationship between gelatin concentration and viscosity was found. This observed nonlinear viscosity behavior can be attributed to the chainlike nature of gelatin particles which leads to increased chain entanglement[23] with increasing concentration. If the gelatin particles were hard spheres an increase in gelatin concentration would result solely in a linear viscosity increase.[24]

Fig. 2 illustrates the effect of differing gelatin sol concentrations on compressive strength of coated HA-ceramics. Increasing the gelatin concentration in the gelatin sols resulted

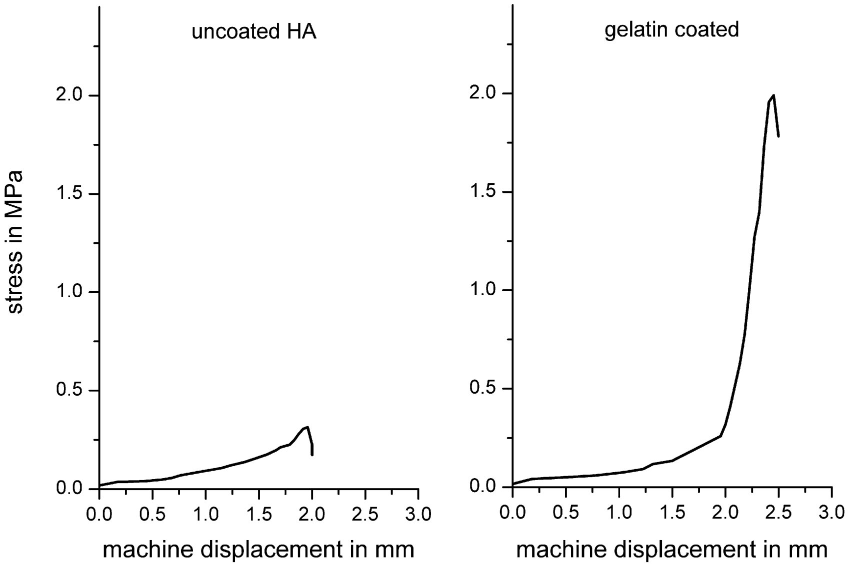

Fig. 3. Stress–displacement curve of uncoated and gelatin coated HA samples ($T(\text{sol}) = 50\,°\text{C}$, $c(\text{gelatin}) = 0.19$ g/g, 250 gbloom).

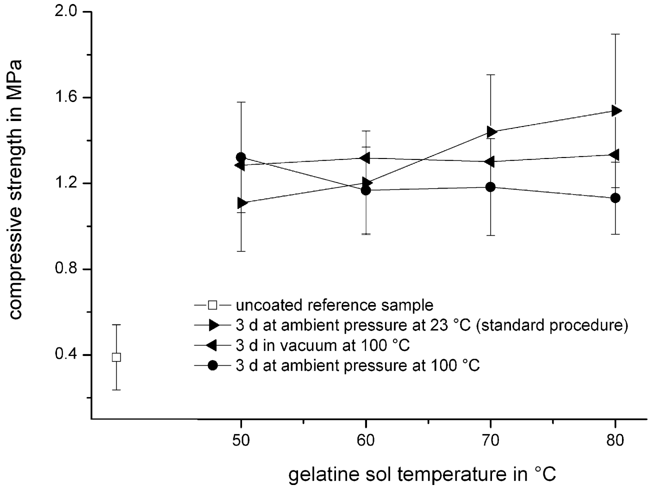

Fig. 4. Effect of gelatine sol temperature and drying regime on compressive strength for gelatin coated (c(gelatin) = 0.07 g/g, 180 gbloom) porous HA-ceramics.

in a rather linear increase in compressive strength. While the uncoated HA-ceramics have a compressive strength of 0.38 MPa, coating with a gelatin sol (0.19 g/g) resulted in a six-fold increase up to 2.22 MPa. Porosity decreased only marginally with increasing gelatin concentration. This implies that the increased gelatin content of the gelatin sols did not markedly increased the total amount of deposited gelatin, which would have otherwise lead to an undesired marked pore blockage.

Only minor differences were observed when choosing gelatins with different gel strengths, i.e. different bloom numbers. This effect can be observed at gelatin concentrations of 0.07 g/g and 0.19 g/g. No significant difference for compressive strength was found when using gels with 80, 180 or 250 gbloom at these particular gelatin concentrations.

After coating with higher concentrated gelatin sols the samples appeared to have been plastically deformed at their bottom and top sides where they were in contact with the clamps of the testing machine. This behavior is reflected in the increased strain until failure for gelatin coated samples as shown in Fig. 3. Pore free HA has a modulus of elasticity of 114 GPa[25] and a tensile strength of 60 MPa.[26] Due to the high porosity the porous HA ceramics had a low compressive strength (∼0,39 MPa).

The results in Fig. 2 refer to gelatin sols with a temperature of 50 °C. No significant difference in compressive strength was found when coating the HA-ceramics with sols having higher temperatures. This can be seen in Fig. 4, which also shows that an additional heat treatment at 100 °C (either in vacuum or at atmospheric pressure) after the initial drying step (3 days at room conditions) did not significantly change the compressive strength.

Fig. 5 shows a fracture surface of a gelatin coated porous HA-ceramic. As can be seen, the strut is hollow. This is a well known feature for ceramics which have been prepared with the foam replication technique.[2] The void inside the strut corresponds to the space which was previously occupied by the supporting polymer sponge. A light microscopy micrograph of a used polymer sponge is shown in Fig. 6.

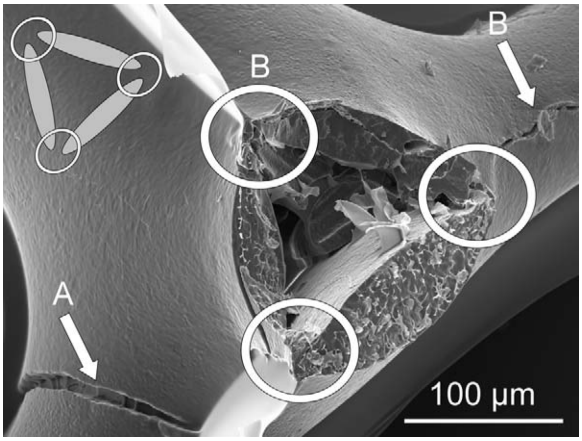

Fig. 5. SEM micrograph showing characteristic features on a fracture surface of a gelatin coated (c(gelatin) = 0.07 g/g, T(sol) = 50 °C, 180 gbloom) HA-ceramic. Arrow A (B) mark *transversal* (*longitudinal*) cracks. The inset drawing illustrates the fact that the struts consist of single strut segments.

The depicted strut in the SEM micrograph in Fig. 5 is not only hollow but a closer SEM observation also revealed (micrograph not shown) that the three lath-like strut segments are not firmly connected but are separated. The occurrence of three separated strut segments is schematically depicted in the inset drawing, which shows a strut whose three laths are disconnected in the encircled regions. Laths become separated due to non-sufficient wetting of the polymer sponge as the fin-like edges, which can be seen in Fig. 6, are hard to cover with a coherent ceramic slurry layer. Two different types of flaws were found which are schematically depicted in Fig. 7. *Transversal* cracks (marked A in Fig. 5), traveling perpendicular across a strut and *longitudinal* cracks (marked B in Fig. 5) going alongside the strut.

The applied gelatin coatings lead to a sealing of longitudinal and transversal cracks as is indicated in the SEM micrograph in Fig. 5. It can clearly be seen that the transversal crack (marked A) and the longitudinal crack (marked B) are sealed by gelatin.

Regarding the sealing of transversal cracks an interesting finding is shown in Figs. 8 and 9. Both micrographs show fracture surfaces of formerly gelatin sealed transversal cracks. While the coating in Fig. 8 was prepared with a highly concentrated

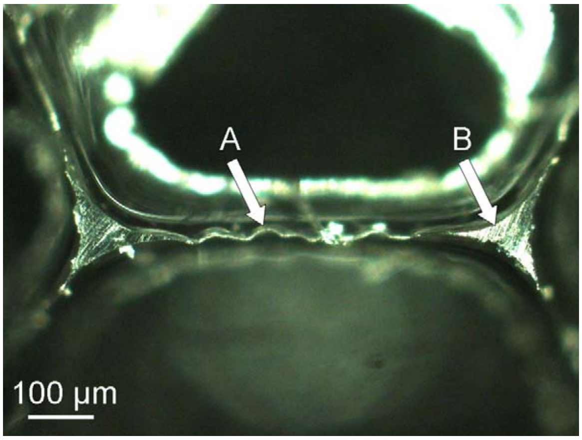

Fig. 6. Light microscopy image of a strut of the used polyurethane sponge. Note the fin-like edges (A) and the triangular cross section (B) of the strut.

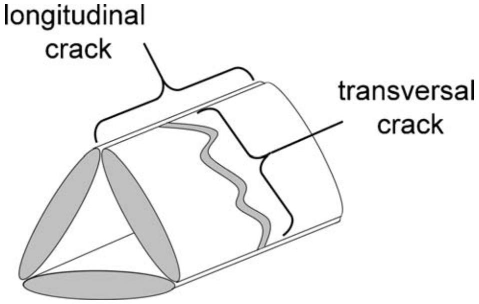

Fig. 7. Schematic drawing of different failure modes (longitudinal and transversal cracks) encountered in porous HA-ceramics prepared with the foam replication technique.

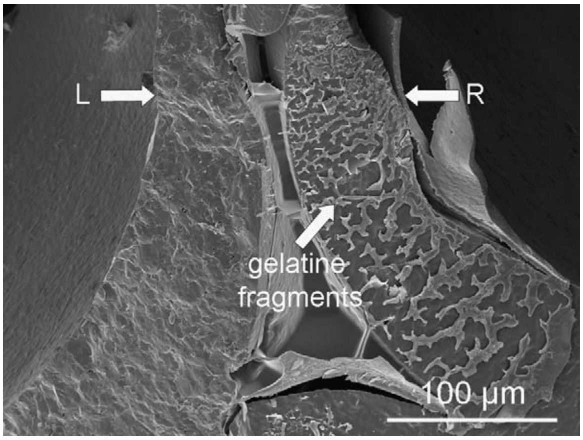

Fig. 8. Transversal crack sealing with applied gelatin coating. SEM micrograph of gelatin fragments on a fracture surface of a transversal crack. The HA-ceramic had been coated with a highly concentrated gelatin sol (c(gelatin) = 0.19 g/g, T(sol) = 50 °C, 180 gbloom). Note the rather big size of the gelatin fragments.

gelatin sol having a concentration of 0.19 g/g the coating in Fig. 9 was prepared with a much more dilute gelatin sol, i.e. 0.07 g/g. If comparing both micrographs it becomes clear that the less concentrated gelatin sol provoked the formation of fewer and much smaller gelatin bridges sealing the transversal crack.

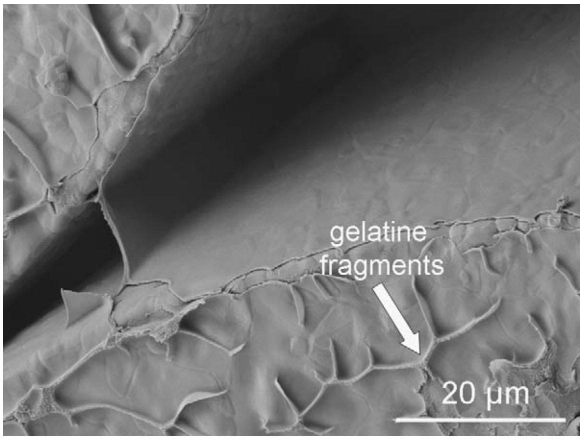

Fig. 9. SEM micrograph of gelatin fragments on a fracture surface of a HA-ceramic which had been coated with a rather dilute gelatin sol (c(gelatin) = 0.07 g/g, T(sol) = 50 °C, 180 gbloom). Note that the gelatin fragments are rather small in size.

4. Discussion

4.1. Influence of gelatin concentration

The present results are in line with earlier works[11,27] reporting that gelatin coatings increase the toughness of porous coated ceramics. This toughening effect can be attributed to crack bridging facilitated by the applied gelatin coating. Crack bridging was discussed by Pezzotti and Asmus[28] who increased the bending strength of hydroxyapatite by injecting nylon-6 and poly(methylmethacrylate) into the pores. Crack bridging was also investigated by Peroglio et al.,[6] who coated alumina scaffolds with PCL. These authors also concluded that the polymer (PCL) acts like a bridging element for cracks.

Two different types of cracks were found in the present work, i.e. longitudinal and transversal cracks. Albeit gelatin coatings bridged both cracks, as can be seen in the fracture surface micrograph Fig. 5, transversal cracks had apparently the most dominant effect. This assumption is based on the following observation. The gelatin sealing of longitudinal cracks remained rather unaffected when using more concentrated gelatin sols. Transversal cracks on the other hand got filled more and more with gelatin material the higher the concentration of the used gelatin sol. When taking a closer look at the gelatin fragments shown in Figs. 8 and 9 one can see that the size of these fragments increases with increasing gelatin concentration. The following scenario is envisaged. During infiltration with gelatin sol the transverse cracks of a strut are completely filled by the liquid gelatin sol irrespective of concentration and bloom number. This assumption is supported by the finding that gelatin could easily enter the cracks within the ceramic coatings as gelatin coating fragments were also found within the hollow struts. After infiltration, probably all transverse cracks were completely filled with gelatin sol. At this stage the gelatin is in its gel state, i.e. contains a lot of water. When these gel-like gelatin coatings – or rather fillings – are dried, the gel-like gelatin material shrinks. Hence, the higher the gelatin concentration of the gelatin sol the more dried gelatin material remains within the transverse cracks. This effect is clearly demonstrated in Figs. 8 and 9.

4.2. Influence of gelatin sol temperature, bloom number and drying regime

The toughening effect was unaffected by differing processing temperatures (50, 60, 70 or 80 °C), hence viscosity, which reduces markedly with increasing sol temperature, does not have much influence on the toughening effect. The limited influence of changing gelatin sol viscosities can also be deduced from the result that differing bloom numbers, which also markedly influence viscosity, do not significantly effect the toughening. As a rule of thumb it can be said that an increased viscosity leads to a higher coating thickness. However, judging the coating thickness proved extremely difficult in the present work. It can be seen in Fig. 8 that the right hand side strut perimeter (arrow R) is coated with a ∼5 µm thick gelatin layer while there is almost no visible coating on the left hand side (arrow L). These differences are probably caused by the removal of excessive gelatin sol by spinning in the centrifuge. However, it should be noted at this point, that an influence of coating thickness on the toughening effect must not be ruled out. By applying a very thick coating (results not shown) onto the HA-ceramics, this was done by immersing a HA-ceramic completely in a gelatin sol and subsequent gelling, it was found that the compressive strength increased up to ∼4 MPa. However, the open porosity disappeared almost completely which was not desired at all.

The investigated different drying regimes do not have a significant influence on compressive strength. It is well known that the mechanical properties of gelatin films depend markedly on water content.[18] As a result, one might have expected an increased toughening effect for coated ceramics after drying at elevated temperatures. However, Yakimets et al.[18] reported that below 12 wt% water content no further increase in tensile strength could be detected. Thus, one might hypothesize that the water content in the gelatin coatings of the air dried ceramics was already so low that any further water removal did not result in an increase in tensile strength. This assumption is supported by weight loss measurements (results not shown in this work). The weight loss of gelatin coating fragments measured with thermal gravimetry ranges at 14 wt% (80 and 250 gbloom, preparation temperature 50 °C, dried at room temperature before thermal analysis). This value for water content is in line with values reported in,[17] and is of similar value as that one (12 wt%) reported by Yakimets et al.[18] below which no further increase in tensile strength can be expected. Hence, a further reduction in water content due to drying for 3 days at elevated temperatures did not result in an increase in compressive strength.

5. Conclusion

Open porous ceramics which were prepared by the foam replication technique observe two types of strut failures, namely longitudinal and transversal cracks. When ceramics having these cracks were coated with gelatin, an increase in compressive strength was observed which can be attributed to crack bridging provided by the applied gelatin coating. It was found that coatings prepared with higher concentrated gelatin sols lead to higher compressive strength values. This effect could be attributed to the increased amount of remaining gelatin material within transversal cracks after gelatin drying. Using gelatin with differing gel strengths (bloom number) as well as applying gelatin sols with different temperatures has only little effect on compressive strength of the coated ceramics. Differing drying regimes, i.e. increasing temperature to 100 °C or putting samples under vacuum, do not provoke changes in compressive strength.

Acknowledgements

The authors would like to thank Aap Biomaterials GmbH for the kind supply with the HA-powder. Special thanks go to S. Benemann for the SEM micrographs. The helpful discussions with B. Fedelich are gratefully acknowledged as well as the support by K. Kittler.

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