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Composition dependent mechanical behaviour of S53P4 bioactive glass putty for bone defect grafting

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Abstract

To improve the handling properties of S53P4 bioactive glass granules for clinical applications, bioactive glass putty formulations were developed. These formulations contain both granules and a synthetic binder to form an injectable material that is easy to shape. To explore its applicability in load-bearing bone defect grafting, the relation between the putty composition and its mechanical behaviour was assessed in this study. Five putty formulations with variations in synthetic binder and granule content were mechanically tested in confined compression. The results showed that the impaction strains significantly decreased and the residual strains significantly increased with an increasing binder content. The stiffness of all tested formulations was found to be in the same range as the reported stiffness of cancellous bone. The measured creep strains were low and no significant differences between formulations were observed. The stiffness significantly increased when the samples were subjected to a second loading stage. The residual strains calculated from this second loading stage were also significantly different from the first loading stage, showing an increasing difference with an increasing binder content. Since residual strains are detrimental for graft layer stability in load-bearing defects, putty compositions with a low binder content would be most beneficial for confined, load-bearing bone defect grafting.

Keywords: Bioactive glass; putty; confined compression; micro computed tomography; load-bearing bone defect grafting

1. Introduction

Large bone defects can arise from trauma, infection, bone tumour removal or other clinical related causes. These defects typically require grafting treatments in order to heal. Such treatments are

common and have a high success rate and therefore bone graft transplantation is the second most frequent tissue transplantation worldwide (Campana et al. 2014). In the case of an infection of the bone and bone marrow, osteomyelitis, not only grafting of the defect is required, but also the eradication of the infection (Calhoun et al. 2009; Sanders & Mauffrey 2013).

Currently, a two-stage surgery treatment is provided to osteomyelitis patients. First, the infected bone is debrided and local and systemic antibiotics are administered. Second, a subsequent surgery is needed to remove the local antibiotics and reconstruct the bone defect with a bone graft or bone graft substitute material (Walenkamp et al. 1998; Sanders & Mauffrey 2013; Chadayammuri et al. 2015). The gold standard material for bone reconstructions is autologous bone. Drawbacks of autologous bone; however, are the donor site morbidity and the quantity and quality of the available donor bone. Several (synthetic) bone graft substitute materials have been proposed, each containing its own specific advantages and disadvantages (Campana et al. 2014; Polo-Corrales et al. 2014; Oryan et al. 2014).

One of these substitute materials is S53P4 bioactive glass in granular form, which is gaining support by surgeons because of the possibility to treat the infection and graft the bone in a single operation (Vugt et al. 2016; Aurégan & Bégué 2015; van Gestel et al. 2015; Baino et al. 2016). Bioactive glass granules have both bone bonding and antimicrobial properties, arising from surface reactions when the granules get in contact with body fluids (Hench 2006; Andersson et al. 1990; Drago et al. 2013; Munukka et al. 2008; Leppäranta et al. 2008; Zhang et al. 2010; Waselau et al. 2012; Välimäki & Aro 2006; Aitasalo et al. 2001). At first contact with body fluid, ions will be released that locally increase the pH and osmotic pressure, which has been related to the antibacterial properties (Munukka et al. 2008; Leppäranta et al. 2008; Stoor et al. 1998). In addition, the ion release causes the formation of a silica layer at the surface of the glass granules that will induce calcium phosphate precipitation, which will eventually crystallise into natural hydroxyapatite that provides bone bonding possibilities (Jones 2013). Osteomyelitis commonly occurs in long bones and therefore not only the antibacterial and bone bonding properties are of importance, but also the load-bearing capacity of the material, especially if the treated defect is located in the lower extremities. From earlier studies, it is known that bioactive glass granules can withstand considerable compressive loading in confined conditions, while the bioactive glass graft layer can be similar to that of cancellous bone (Hulsen et al. 2016).

A drawback of applying loose S53P4 bioactive glass granules is their suboptimal handling properties (Peltola et al. 2008). For this reason S53P4 bioactive glass putty formulations have been developed, which contain bioactive glass granules of two different sizes that are carried by a synthetic binder, consisting of polyethylene glycol (PEG) and glycerol. These putty formulations provide a composite material that is both formable and injectable. While the composition of the bioactive glass granules in the putty was not changed, the mixture of different granule sizes and the addition of the binder could considerably change the mechanical properties of the graft layer compared to those measured for the granules alone (Hulsen et al. 2016; Iviglia et al. 2016). The aim of this study was therefore to determine the composition dependent impaction, creep, and residual strains and aggregate modulus in confined compression. These parameters could provide knowledge of the graft material for the possible application in load-bearing bone defect grafting, in which graft layer stability is highly important. Dynamic confined compression tests were performed similar to those used in earlier studies on bone graft substitute materials (Walschot et al. 2010; Hulsen et al. 2016). To study the relation between the formulation of the putty and the mechanical properties, the composition of the putty was varied in synthetic binder and glass granule content.

2. Materials and methods

2.1 Sample preparation

Five different formulations of BonAlive® putty (obtained from BonAlive Biomaterials Ltd., Turku, Finland) with increasing synthetic binder contents, were tested in confined compression tests to determine the relation between the mechanical behaviour of the putty and its composition (n=5 for all groups). These putty formulations consisted of a synthetic binder, S53P4 bioactive glass granules (2-3.015 mm; Figure 1), and S53P4 powder (0.5-0.8 mm) in different mixtures (Figure 2). The binder consisted of PEG 400 (20 wt%), PEG 1500 (40 wt%), PEG 3000 (15 wt%) and glycerol (25 wt%) and the bioactive glass had the specific composition of 23.0 wt% Na₂O; 20.0 wt% CaO; 4.0 wt% P₂O₅ and 53.0 wt% SiO₂.

Before confined compression tests were performed, the samples were impacted using a custom-made impaction device, to obtain clinically relevant impaction strains, as previously described (Hulsen et al. 2016; Walschot et al. 2010). The height of each sample was measured prior to impaction, represented as h_{init} , and after impaction, represented as h_0 . The impactability of the sample was defined as:

$$Impactability = -\varepsilon_{imp} \cdot 100\% = -100\% \cdot \ln\left(\frac{h_0}{h_{init}}\right) \quad (1)$$

2.2 Confined compression tests

After impaction all samples were subjected to dynamic confined compression tests using a biomaterials testing system (858 Mini Bionix®, MTS Systems Corporation, Eden Prairie, MN, USA). The loading regime consisted of two dynamic loading phases and two resting phases and was based on the loading regime as previously described (Hulsen et al. 2016; Walschot et al. 2010). Briefly, during the dynamic loading phases the samples were subjected to 900 sinusoidal loading cycles (40-850 N at 1 Hz) followed by 300 seconds of rest (unloaded). The second loading phase was identical and executed directly after the first resting phase. A schematic representation of the loading regime is shown in Figure 3.

The displacement and force were recorded and the height of the sample at different stages of the experiment was calculated. Based on these heights, three true strain measures were calculated. First, the residual strain ($\varepsilon_{residual}$), which was defined as the logarithmic strain calculated from the difference in sample height measured after the end of the resting phase (h_{unload}) and after impaction (h_0):

$$\varepsilon_{residual} = \ln\left(\frac{h_{unload}}{h_0}\right) \quad (2)$$

Second, the creep strain was defined as the logarithmic strain calculated from the height decrease during loading that was restored after the corresponding unloading phase:

$$\varepsilon_{creep} = \ln\left(\frac{h_{min}}{h_{unload}}\right) \quad (3)$$

Third, the cyclic elastic strain was calculated as the average strain in the last 50 loading cycles (Eq. 4). Together with the stress difference in one loading cycle (Eq. 5) the cyclic strain was used to calculate the aggregate modulus (H, Eq. 6), as a measure for the stiffness of the material:

$$\varepsilon_{elastic} = \ln\left(\frac{h_{max}}{h_{min}}\right) \quad (4)$$

$$\sigma_{elastic} = \frac{\Delta F_{cyc}}{A_{sample}} \quad (5)$$

$$H = \frac{\sigma_{elastic}}{\varepsilon_{elastic}} \quad (6)$$

2.3 Microcomputed tomography analysis

To gain insight into the content distribution and possible damage in the granules after confined compression of the five formulations, microcomputed tomography (microCT) images were obtained at the end of the experiment (μ CT80, SCANCO Medical AG, Brüttisellen, Switzerland). The parameters used for scanning involved an isotropic voxel size of 36 μ m; an energy of 70 kVp; a current of 114 μ A; and an integration time of 300 ms. Based on the histograms of the attenuation coefficients, thresholds were defined to segment the glass and the binder from the images. The threshold range for bioactive glass granules was set to 808-3015 mgHA/ccm, for the binder a threshold of 288-807 mgHA/ccm was used and the remaining voxels (in range of 0-287 mgHA/ccm) were assumed to be air-voids. In addition, the segmented bioactive glass granules were analysed to investigate the amount of granules that are in direct contact with each other, using a component labelling algorithm. Finally, the size of the granules was determined using a distance transform algorithm to investigate if any granule fractures after impacting and confined compression occurred. All analyses were performed using IPLFE v02.01 (Scanco Medical AG, Brüttisellen, Switzerland).

2.4 Statistical analysis

Statistical analysis was performed with SPSS® (IBM® SPSS® Statistics Version 22, SPSS Inc. Chicago, USA). Levene's test was used to test the homogeneity of variances and a Shapiro-Wilk test of the studentized residuals was used to evaluate the assumption of normality. For comparison of the composition dependent parameters, ANOVA followed by Tukey's post hoc test was performed if homogeneous variances and a normal distribution could be assumed for the analysed parameter. If no normality could be assumed a Kruskal-Wallis test was performed, and if normality could be assumed with unequal variances the Welch's test with a Tamhane's T2 post hoc test was executed.

To test the difference between the two loading stages, a pair-wise comparison was performed. For this analysis either a paired t-test or a nonparametric related samples test was performed, depending on the assumption of normality (determined by Shapiro-Wilk analysis). In all tests, significance was assumed for a p-value < 0.05.

3. Results

The results (mean $\pm$ standard deviation) per loading and resting stage are shown in Table 1 and 2. Significant differences between the two loading stages are represented by asterisks in Table 2, which show that especially the aggregate modulus and the residual strains are significantly different in the second loading stage, compared to the first. The results with significant differences between the five putty formulations for loading stage 1 are shown in Figure 4. Significant differences between the putty formulations are observed for the impactability and for the residual strains. The putty samples with a lower binder content (putty B) shows a significantly higher impactability and significantly lower residual strain than the putty samples that contained a higher binder content (putty E). This high amount of binder containing formulation has statistically higher residual strains than all other formulations, which contain less binder. In the boxplots the samples that exceed 1.5 times the interquartile ranges are indicated, but these are not assumed to be outliers. For the residual strains one sample (of putty E) was indicated as an outlier, as the height measurement of the biomaterials testing system failed and therefore h_0 could not be determined from the data. Since the other parameters were not influenced by h_0 they were yet analysed.

MicroCT data analysis show that the average air-void content detected in the putty samples never exceeded 15 %. The component labelling results demonstrate that over 98% of the granules make contact after mechanical loading. The amount of granules that were labelled as being in contact slightly decreased with an increasing binder content, being 99.7 ± 0.09 % of the granules in putty A and 98.6 ± 0.16 % of the granules in putty E. The distance transform distributions (Figure 5) show a slight decrease in measured granule size with decreasing synthetic binder content; although, no significant difference was found for the mean granular size.

4. Discussion

Bioactive glass granules have gained interest from clinicians because of their antibacterial and bone bonding properties. The granules are able to withstand high compressive forces (Hulsen et al. 2016), but they do not possess appropriate handling properties. To overcome this practical issue, bioactive glass putty formulations have been developed. This study aimed to investigate whether this newly-developed composite material is also able to withstand high compressive loads, which may enable the application of the material in load-bearing bone grafting. Therefore, confined compression tests were performed on five different formulations of the putty, which allows determination of the relation between mechanical behaviour and the putty composition.

Whereas other injectable formulations have been developed based on bioceramics such as calcium phosphate or hydroxyapatite (Iviglia et al. 2016) that may potentially also withstand high compressive forces, these lack the antibacterial properties of bioactive glass. For the present putty, these antibacterial properties still need to be confirmed. However, since PEG and glycerol are water soluble, the binder is expected to dissolve in a physiological environment. To the author's knowledge, there is only one paper mentioning the dissolving time of a similar binder in a similar putty. Wang et al. (2011) report a degradation time of 48-78 hours for a putty containing 45S5 bioactive glass with a binder containing PEG and glycerol (Wang et al. 2011). This indicates a fast dissolution rate, hence, exposure of the bioactive glass surface with its antibacterial properties. For this reason, the putty might be of particular interest in infected load-bearing applications, such as management of prosthetic joint infections (Arts & Geurts 2017).

The largest significant differences between the compositions, were found in residual strains. With an increasing synthetic binder content the residual strain increased significantly. The putty that contained the highest binder content showed similar residual strains as observed for bone morsels by Hulsen et al. (2016) (Hulsen et al. 2016). These strains were calculated from deformations that are indicated to be irreversible, since the material did not restore that height decrease during the unloading phase of the confined compression test. The permanent deformations measured in this study are most likely due to binder loss, as it was observed that the binder was pressed out of its confined environment. The binder composed of PEG and glycerol has a viscous nature and was found in and around the brass filter, which was placed on top of the samples in the test setup. For samples that contained a higher binder content more expelled binder was found, which explains the higher residual strains in these groups. It is likely that the residual strains originate from the binder and not from the granules, as the granules themselves showed low residual strains in a previous study (Hulsen et al. 2016). The residual strains observed after the first stage, are likely to be responsible for the significant differences in stiffness and residual strains observed in the second loading stage. Since the residual strains indicate permanent deformations, these strains will affect graft layer stability when used in load-bearing defects and therefore should kept as low as possible. Although, this will lead to less convenient handling properties.

Also creep strains will affect graft layer stability in load-bearing applications. The creep strains for the different putty formulations were found to be relatively low compared to bone morsels, but higher than the creep strains reported for loose bioactive glass granules (Hulsen et al. 2016). In terms of creep behaviour, this indicates that the putty, independent of its formulation, might be mechanically more suitable in load-bearing applications than morselized bone from autografts or allografts. No significant differences were observed between the different putty formulations.

Since it is often the cancellous bone that is replaced by the bioactive glass, the stiffness of the construct should be representative for cancellous bone. The stiffness of cancellous bone is reported to be in range of 100-500 MPa (Ratner et al. 2012). The observed stiffness in our study, represented as the aggregate modulus, was in this range for all putty formulations and based on this parameter they can be considered appropriate as bone graft substitutes. This stiffness is lower than the stiffness reported for bioactive glass granules that have a stiffness of 653 MPa (Hulsen et al. 2016; Ratner et al. 2012). The latter is slightly higher than the range in stiffness reported for cancellous bone, which may lead to stress shielding (Huiskes et al. 1992).

Impactability, which is related to the impaction strains is low compared to other reported materials (Hulsen et al. 2016; Walschot et al. 2010; Verdonchot et al. 2001). According to Kerboull et al. (2009), the impaction is important in order to reduce the graft layer strains (Kerboull et al. 2009). Both Walschot et al. (2010) and Hulsen et al. (2016) measured the impaction strain for bone morsels, but although the described methods seem similar, there is a large difference in reported values between the two studies (0.46 ± 0.01 vs. 0.92 ± 0.06 , respectively) (Hulsen et al. 2016; Walschot et al. 2010). The impaction strains observed for the five putty formulations were lower than these reported values.

MicroCT analysis showed an air-void content of maximal 15%. This indicates that the binder filled most of the space between the granules, after impaction and mechanical testing. Component labelling showed that most of the voxels that were segmented as bioactive glass granule belonged to one component, which indicates that all granules were in contact with one another, and that the binder thus is located only in the voids between granules. Due to the limited resolution of the microCT images (36 μm), it is possible that binder layers between granules that are less than 36 μm were not detected, in which case contact regions would be overestimated. However, such a thin layer would not affect the stiffness of the sample much and merely act as a lubricant. Distance transform analysis of the microCT images showed a slightly lower granule size distribution for samples with a low synthetic binder content compared to samples with a high binder content. This suggests that some of the granules in the samples with low binder content were damaged during impaction or testing. Unfortunately no images were obtained before testing, but since the putty formulations were fabricated according to the contents that are visualized in Figure 2 and since the granules in the five formulations all originated from the same granule batches (two sizes) it seems unlikely that initial differences in size between the groups could explain these results.

Some limitations of the present study should be discussed as well. First, the material properties were measured only for confined compression conditions, in which case no shear deformations are applied. For granular materials, the resistance to shear loading typically is very low, thus limiting the results presented here to the confined compression case. However, in clinical practice bioactive glass putty formulations will be used in a more or less confined environment. In the treatment of osteomyelitis in peripheral bones, a cortical window is created in order to be able to debride and graft the defect, the remaining cortical bone will then ensure a more or less confined environment. If confinement during impaction grafting cannot be realised the load carrying properties of the putty will likely be very low.

Second, the material properties found are depended on the number of loading stages. Significant differences in aggregate moduli and residual strains were found between the two stages. The residual strains in the first loading phase that resulted from a permanent decrease in height, ensured a slight, but significant, increased stiffness in the second loading phase. This can be explained by the loss in binder, since bioactive glass granules alone were found to be even stiffer (Hulsen et al. 2016). Although significant, differences in material properties measured for the first and second stage were small, e.g. the difference in aggregate modulus was less than 5.5%. Although applying more loading stages could further change the properties, differences are expected to be even smaller then. Unfortunately, the direct translation of the loading stages to the *in vivo* situation is not possible since the loading regime of the material in an *in vitro* environment will be different than the *in vivo* situation (Dalstra & Huiskes 1995).

Third, the results are valid only for the direct post-operative situation. As mentioned above, the binder is expected to dissolve in a physiological environment quite quickly. This fast dissolution rate is likely to affect the mechanical behaviour of the material over time, therefore degradation characteristics should be studied in the future.

5. Conclusion

The direct post-operative mechanical behaviour of bioactive glass putty formulation is related to its composition. A higher synthetic binder content is related to increased residual strains and decreased impactability, but compared to bone morsels (as gold standard), the mechanical behaviour of bioactive glass putty formulations is at least as beneficial for the application in load-bearing defects, in particular for formulations with lower binder content. Load bearing is strictly limited, however, to well confined applications, and it remains to be investigated to what extent the dissolving of the matrix will affect the mechanical properties over time.

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7. Disclosure

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Figures

Graphical abstract

Figure 1. A scanning electron microscopy image of a S53P4 bioactive glass granule.

Figure 2. The contents of the five tested putty formulations. The synthetic binder content increased as the amount of bioactive glass granules content decreased, such that putty A contained the lowest and putty E contained the highest synthetic binder content.

Figure 3. Schematic illustration of the two-stage loading regime used for confined compression tests. The regime contained two dynamic loading phases of 900 cycles and two resting phases of 300 s each. The first resting phase is directly followed by the second dynamic loading phase.

Figure 4. The results of the confined compression tests for the five different putty formulations calculated from loading stage 1 represented in boxplots. Figure a) shows the impactability per formulation, b) the aggregate moduli, Figure c) shows the creep strain and d) the residual strains. The asterisk above the beams indicate a p-value < 0.05 between the two indicated formulations. Note that the small circles and stars indicate the values that are out and far out of the $1.5 \times$ interquartile range as indicated by SPSS®, but they do not denote outliers. The letters A, B, C, D and E on the x-axes refer to the putty formulations as shown in Figure 2.

Figure 5. Distance transform results, represented as mean $\pm$ standard deviation, for the two putty's containing the lowest and highest synthetic binder content.

Tables

Table 1. Results calculated from loading stage 1, represented as mean $\pm$ standard deviation.

Putty	Impactability [%]	H [MPa]	ϵ_{creep} [-]	$\epsilon_{residual}$ [-]
A	27.0 $\pm$ 4.6	181.1 $\pm$ 35.8	-0.013 $\pm$ 0.004	-0.012 $\pm$ 0.004
B	29.8 $\pm$ 4.5	215.7 $\pm$ 19.7	-0.010 $\pm$ 0.003	-0.020 $\pm$ 0.007
C	24.4 $\pm$ 2.1	229.5 $\pm$ 35.7	-0.009 $\pm$ 0.001	-0.031 $\pm$ 0.008
D	21.6 $\pm$ 12.2	239.8 $\pm$ 40.2	-0.012 $\pm$ 0.010	-0.071 $\pm$ 0.024
E	15.8 $\pm$ 2.1	224.2 $\pm$ 24.7	-0.016 $\pm$ 0.006	-0.329 $\pm$ 0.487

Table 2. Results calculated from loading stage 2, represented as mean $\pm$ standard deviation. Significant differences compared to loading stage 1 are labelled with * ($p < 0.05$).

Putty	H [MPa]	ϵ_{creep} [-]	$\epsilon_{residual}$ [-]
A	184.6 $\pm$ 37.7 *	-0.012 $\pm$ 0.004	-0.014 $\pm$ 0.004 *
B	220.7 $\pm$ 21.1 *	-0.009 $\pm$ 0.003 *	-0.022 $\pm$ 0.008 *
C	239.0 $\pm$ 38.9 *	-0.008 $\pm$ 0.001	-0.035 $\pm$ 0.009 *
D	249.5 $\pm$ 39.2 *	-0.009 $\pm$ 0.001 *	-0.078 $\pm$ 0.025 *
E	236.3 $\pm$ 23.9 *	-0.014 $\pm$ 0.005 *	-0.339 $\pm$ 0.120 *