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In vitro study investigating the mechanical properties of acrylic bone cement containing calcium carbonate nanoparticles

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Abstract A successful total hip replacement has an expected service life of 10–20 years with over 75% of failures due to aseptic loosening which is directly related to cement mantle failure. The aim of the present study was to investigate the addition of nanoparticles of calcium carbonate to acrylic bone cement. It was anticipated that an improvement in mechanical performance of the resultant nanocomposite bone cement would be achieved. A design of experiment approach was adopted to maximise the mechanical properties of the bone cement containing nanoparticles of calcium carbonate and to determine the constituents and preparation methods for which these occur. The selected conditions provided improvements of 21% in energy to maximum load, 10% in elastic modulus, 7% in bending strength and 8% in bending modulus when compared with bone cement without nanoparticles. Although cement containing nano$CaCO_3$ coated in sodium citrate also enhanced the energy to maximum load by 28% and the elastic modulus by 14% when compared with control cement, it is not recommended as a factor in the production of nanocomposite bone cement due to reduction in the bending properties of the final bone cement.

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Abbreviations

BPO	Benzoyl peroxide
DDS	Drug delivery systems
DOE	Design of experiments
DMPT	*N*,*N*-Dimethyl-*para*-toluidene
EDX	Energy dispersive X-ray microanalysis
HA	Hydroxyapatite
MMA	Methyl methacrylate
nano$BaSO_4$	Nanoparticulate barium sulphate
nano$CaCO_3$	Nanoparticulate calcium carbonate
PMMA	Polymethyl methacrylate
SA	Stearic acid
SC	Sodium citrate
SD	Standard deviation
SEM	Scanning electron microscopy
SPT	Small punch test
$\bar{X}$	Mean

1 Introduction

Over 43,000 total joint replacements are performed by the NHS annually [1]. Whilst acrylic bone cement is used successfully in orthopaedic surgery, its performance under physiological loading could be improved. Each year 9,500 hip revisions take place in the UK [2] causing further discomfort to the patient and are a drain of valuable health service resources. A typical successful total hip replacement has a service life of 10–20 years with over 75% of failures due to aseptic loosening which is attributed to cement mantle failure [3].

Fibres and particles have been employed to increase the mechanical properties of acrylic bone cement; some more successfully than others. Materials such as conventional polymer systems, carbon fibre, Kevlar®, metal

fibres, hydroxyapatite and bone particles have all been considered [4].

Nanotechnology is rapidly developing with potential to become an essential element in our everyday lives. Nanoparticles have been used for many different applications in biology, medicine, pharmacy and engineering [5–7]. The principal difference between nano-sized particles and their micron-sized equivalents is a greatly increased surface area to volume ratio [8]. In this study, nanoparticles of calcium carbonate (nano$CaCO_3$) were chosen for addition to acrylic bone cement. Recently commercially available and organically modified nanoclays have been added to acrylic based bone cements resulting in significant improvements in mechanical properties [9, 10]. Nanoparticulate barium sulphate (nano$BaSO_4$) has also been incorporated in acrylic bone cement [11–18] to replace micro-sized $BaSO_4$, which acts as a radiopaque agent in the cement. The application of nano$BaSO_4$ reduced the detrimental effects that are associated with using micro-sized radiopaque agent in bone cement such as inferior fatigue performance and high levels of abrasive wear.

$CaCO_3$ particles have previously been added to calcium phosphate cement to liken its structure to bone mineral and reduce its cytotoxicity [19–21]. Research has also been carried out on the feasibility of using $CaCO_3$ nanoparticles in drug delivery systems (DDS). In vivo and in vitro studies have demonstrated the viability of absorbing drug agents onto nano$CaCO_3$ and subsequently characterising their efficacy [22]. The advantage nano$CaCO_3$ has over other DDS is that their smaller size means they can reach regions that other DDS may not, such as inflamed tissues, and can also avoid phagocytosis (process where cells engulf and ingest foreign bodies). Recently, $CaCO_3$ nanoparticles have been added to conventional linear polymers to enhance particular mechanical properties of the final product by tailoring the method of mixing and the percentage loading [23–26].

The aim of this work was to investigate the inclusion of nano$CaCO_3$ in acrylic bone cement. It was anticipated that an improvement in mechanical performance of the resultant nanocomposite bone cements would be observed. A design of experiment (DOE) approach was adopted to analyse the mechanical properties of nanocomposite bone cement and to determine the experimental conditions at which these enhanced characteristics occur.

2 Materials

Colacryl B866 (Lucite International, UK), a methyl methacrylate (MMA) polymer powder and benzoyl peroxide (BPO) (Sigma Aldrich, UK) were used in this study with MMA liquid monomer (Sigma Aldrich, UK) and *N*, *N*-dimethyl-*para*-toluidene (DMPT) (Sigma Aldrich, UK) according to a previous study [9]. Nano-sized precipitated $CaCO_3$ grade CCR with a stearic acid (SA) treatment was used (Yanhua Chemicals, China). The nano$CaCO_3$ had a surface area of 31.26 m^2/g with a particle size range of 40–80 nm. When required sodium citrate (SC) was purchased from Sigma Aldrich (UK) and sodium citrate coated nano $CaCO_3$ was prepared according to a previous study [10]. Coating the nanoparticles in either stearic acid or sodium citrate aids its dispersion throughout the polymer matrix [12, 14, 15, 27].

3 Method

3.1 Design of experiments

A full factorial DOE was completed at two levels (details of the factors and factor levels used are given in Table 1). It had previously been identified by the authors that percentage (by wt) loading of nano$CaCO_3$, method of incorporating the nano$CaCO_3$ into the cement and type of coating used on the nano$CaCO_3$ all have a direct influence on the mechanical properties [28]. As a result, this study was designed to determine suitable factor levels and detect any interactions that may occur. Design-Expert Software, Version 5 was used to analyse the resultant mechanical properties. The non-variable factors in the DOE were humidity, impurities and contamination within the cement, cleanliness during experimentation, consistency of mixing technique and the presence of defects in bone cement test specimens. The constant factors were the base constituents of the polymer powder and liquid monomer, bone cement consistency, mixing system, duration of cement mixing, storage conditions for cement and the preparation technique for manufacture of the cement test specimens. The measurable responses were the energy to maximum load (mJ) elastic modulus (MPa), bending strength (MPa), bending modulus (MPa) and compressive strength (MPa).

Table 1 Details of factors and factor levels used in DOE

Factor	Units	Type	Low	High
Percentage nano$CaCO_3$	% wt	Numerical	0.25	0.75
Mixing type	–	Categorical	Ultrasonic bath (30 min)	Ultrasonic probe (30 s)
Coating	–	Categorical	Stearic acid (SA)	Sodium citrate (SC)

3.2 Mechanical testing

Mechanical assessment of the bone cement produced from each run was performed using the Small Punch Test (SPT) technique, bending testing and compressive testing. The SPT technique [29] characterises the ductility and fracture resistance of metals and polymers. Samples were reduced to a nominal diameter of 6.4 ± 0.05 mm, from which 0.5 ± 0.01 mm discs were produced using an Accutom-50 diamond-cutting saw (Struers A/S, Denmark). The SPT method was conducted using a Lloyds materials testing machine (Lloyds Instruments, UK) at a constant displacement of 0.5 mm min^{-1} to failure. Furthermore, to simulate physiological conditions each SPT was conducted at 37 ± 0.2°C in deionised water. A load versus deformation plot was used to determine the fracture behaviour of the bone cement [29]. Bending and compression testing were conducted using a Lloyds materials testing machine (Lloyds Instrument Ltd., UK) in accordance with ISO 5833. Consequently, the bending strength, bending modulus and compressive strength were obtained.

3.3 Sample preparation

Eight runs were required for the DOE (Table 2). Each run was prepared according to the appropriate factor levels. For example, in Run 1: 0.25% of coated nano$CaCO_3$ (by weight of Colacryl B866) was added to 18.22 g of MMA and placed in a Fisherbrand FB11006 ultrasonic bath (Fisher Scientific, UK) for 30 min. Subsequently, 0.15 g of DMPT was combined with the mixture, which was then added to 36.36 g of Colacryl B866 polymer powder and hand mixed for 40–50 s. For samples mixed using a probe, the MSE Soniprep 150 ultrasonic mixer (Integrated Services TCP Inc., USA) was used on the nano$CaCO_3$–MMA mix for 30 s. The factor levels were selected based on initial experimentation and experience of nanocomposite cements.

Table 2 Run order for full factorial DOE

Run	Percentage nano$CaCO_3$ (% wt)	Mixing type	Coating
1	0.25	Bath	SC
2	0.25	Probe	SA
3	0.75	Probe	SA
4	0.75	Bath	SC
5	0.25	Bath	SA
6	0.75	Bath	SA
7	0.75	Probe	SC
8	0.25	Probe	SC

Data collated for all experimental tests were evaluated for statistical significance using a one-way analysis of variance with P-value <0.05 denoting significance. Post hoc tests were conducted using the Student–Newman–Keuls and Duncan methods. All the tests were conducted using commercially available software (InStat 3.06; GraphPad Software, USA).

4 Results and discussion

4.1 Mechanical testing

Table 3 shows the DOE mean values, $\bar{X}$, ($n = 8$) with standard deviation, SD, obtained from the different mechanical tests conducted. The bone cement produced by Runs 1, 2, 5 and 8 demonstrated an energy to maximum load significantly higher (P-value < 0.05) than control cement, with the cement mixed as per Run 8 having a mean energy to maximum load 28.4% greater than the control value. Run 8 (P-value < 0.05) also showed the largest elastic modulus at 2,531 ± 188 MPa, which is an increase of 14.0% when compared with the control cement. Runs 1, 2 and 8 all exhibited higher elastic moduli than control cement.

There was negligible difference (3.7 MPa) in bending strength for the bone cement produced in Runs 1, 2, 3, 6 and 7, with Run 2 achieving the highest bending strength at 80.3 ± 4.7 MPa, which was 6.5% greater than the control cement. Runs 5 and 8 exhibited the lowest bending strengths of ca. 66.3 MPa, approximately 12% lower than the control cement. Furthermore, the cements prepared by Runs 5 and 8 demonstrated the lowest bending modulus values. Run 4 produced the largest bending modulus which at 3,376 ± 278 MPa was ca. 17% higher than control cement (P-value < 0.05). As with the bending strength data, the cement specimens prepared as per Runs 1, 2, 3, 6 and 7 displayed a marginal difference in bending modulus, which were of similar value to the modulus of the control cement. The mean compressive strength values determined for the nanocomposite cements were consistently less than the control cement (63.4 ± 1.9 MPa). However, no significant reduction (P-value > 0.05) in the compressive strength of the nanocomposite cements produced by Runs 1, 2, 3, 7 and 8 was noted.

From the mechanical testing data it can be observed that many of the DOE runs performed well. Run 8 produced cement that demonstrated the highest energy to maximum load and elastic modulus albeit its bending strength and bending modulus were low in comparison to the other runs. The cement prepared by Runs 2 and 4 exhibited the highest bending and strength and modulus and the cement that displayed the greatest compressive strength was produced by Run 1. From Table 2 it can be observed that no one

Table 3 Summary of mean mechanical testing results (with SD) for DOE ($n = 8$)

	Energy to maximum load (mJ)		Elastic modulus (MPa)		Bending strength (MPa)		Bending modulus (MPa)		Compressive strength (MPa)	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Run 1	36.5	2.4	2,366	182	80.2	3.6	3,042	406	61.9	1.3
Run 2	39.1	5.9	2,431	64	80.3	4.7	3,090	151	58.5	1.0
Run 3	28.4	3.3	2,154	215	79.2	5.0	2,963	129	58.4	2.0
Run 4	30.3	2.2	2,051	129	73.2	14.7	3,376	278	55.4	2.6
Run 5	36.5	3.1	2,197	319	66.6	0.8	2,614	239	54.4	3.2
Run 6	31.4	1.4	2,149	171	77.1	4.5	3,099	132	55.4	1.1
Run 7	32.9	5.6	2,128	303	76.6	6.0	3,000	265	59.8	1.3
Run 8	41.5	7.4	2,531	188	66.3	11.1	2,628	294	60.1	2.2
Control	32.3	5.4	2,220	201	75.4	3.4	2,875	165	63.4	1.9

factor level is the same for Runs 1–8 therefore different mechanical properties are affected by different factors. Thus, the design-expert analysis software was used to provide a more detailed assessment.

4.2 Design of experiments

Table 4 displays output data from the design-expert analysis software showing the percentage contribution (over a threshold of 10%) that the various factors have on each mechanical property. Percentage of $nanoCaCO_3$ added to the bone cement is the predominant factor affecting energy to maximum load and elastic modulus, although mixing method does have a small influence over elastic modulus. The bending strength is governed by all of the factors with an interaction between the three factors accounting for the largest proportion of the changes in bending strength. It was also found that all factors affected the bending modulus, although with different outcomes. The bending modulus factor levels are contradictory to the bending strength results, the most suitable factor levels are 0.75% (wt) $nanoCaCO_3$ coated with SC mixed using an ultrasonic bath. All factors influence the compressive strength with the largest contribution recorded being for the type of coating of the $nanoCaCO_3$.

The percentage differences between the best factor levels and those which had a detrimental effect for each mechanical property are listed in Table 5. The largest percentage difference occurs for energy to maximum load which changes by ca. 25% when the factor levels are altered. In contrast there is a much smaller variation in compressive strength (9%) when factor levels are changed. The coating material had the greatest influence on the bending modulus and compressive strength in favour of using SC. While the bending strength favours the use of SA and the energy to maximum load and elastic modulus do not change when using either coating. Considering this and referring to Table 5, using SC coating would produce a greater improvement in mechanical properties when compared to coating with SA.

To verify this, a comparison between cement coatings was conducted. If the optimal conditions were to contain

Table 4 Percentage contribution value of factors influencing response variables for DOE

Response variable	Factor	Contribution (%)	Preferred factor level
Energy to maximum load	A	80.9	0.25% (wt)
Elastic modulus	A	67.3	0.25% (wt)
	B	14.3	Probe
Bending strength	A, B, C	45.6	0.25% (wt), probe, SA coating
	B, C	37.8	Probe, SA coating
Bending modulus	B, C	35.9	Bath, SC coating
	A	31.7	0.75% (wt)
Compressive strength	C	28.6	SC coating
	B	23.3	Probe
	A, C	14.5	0.25% (wt), SC coating
	A, B, C	13.0	0.25% (wt), probe, SC coating

Table 5 Percentage difference between highest possible and lowest levels in the designed experiment using design-expert point predictor

	Difference (%)
Energy to maximum load	24.8
Elastic modulus	18.5
Bending strength	20.7
Bending modulus	23.7
Compressive strength	9.1

Table 6 Percentage difference for mechanical properties of bone cement prepared as per Runs 2 and 8 when compared with control cement

	Percentage comparison with control	
	Run 2 (SA) (%)	Run 8 (SC) (%)
Energy to maximum load	+21.0	+28.4
Elastic modulus	+9.5	+14.0
Bending strength	+6.5	−12.1
Bending modulus	+7.5	−8.5
Compressive strength	−7.8	−5.14

nanoCaCO$_3$ coated in SC, it would have the same factors as Run 8 and if it were to have nanoCaCO$_3$ coated in SA, the conditions would be the equivalent to Run 2. Reviewing the results (as listed in Table 6), Run 2 has a higher bending strength and modulus, Run 8 has a higher energy to maximum load and elastic modulus and both have similar compressive strengths.

A further analysis of the difference between the coatings of the nanoparticles was completed with scanning electron microscopy (SEM) using the backscattering function where electrons are emitted depending on the material's atomic number. This highlights different materials present in a sample which enables a clearer image of the dispersion and proportion of nanoCaCO$_3$ on the fracture surface. Figure 1a shows an image of the fracture surface of nanocomposite bone cement with nanoCaCO$_3$ coated in SA while Fig. 1b is for the nanocomposite cement with nanoCaCO$_3$ coated in SC. The white particles are agglomerations of nanoCaCO$_3$ which was confirmed using energy dispersive X-ray microanalysis, as displayed in Fig. 2. Cement containing nanoCaCO$_3$ coated in SA had an improved dispersion of nanoCaCO$_3$ with a smaller agglomeration size when compared with cement with CaCO$_3$ coated in SC. SA and SC have both been successfully used before as coating agents in previous studies [12, 14, 15, 27] but not with the specific materials and experimental conditions carried out in this research. The presence of a larger proportion of nanoCaCO$_3$ agglomerations could be the cause of the differences in bending strength and bending modulus between cements with different nanoparticulate coatings, as seen in Table 6.

Considering this, and the time required to coat the nanoCaCO$_3$ in SC, the enhancement shown on the mechanical properties is not justified when compared to nanoCaCO$_3$ coated in SA. Using an ultrasonic probe provided the most efficacious method of mixing the nanoCaCO$_3$ into the liquid monomer constituent of the bone cement. Not only did the probe provide improved mechanical properties but the process only takes 30 s, in comparison with mixing in an ultrasonic bath which requires 30 min. It is postulated that the increase in mechanical properties is due to the greater dispersion of nanoCaCO$_3$ obtained by the ultrasonic probe. Table 7 shows the recommended factor levels.

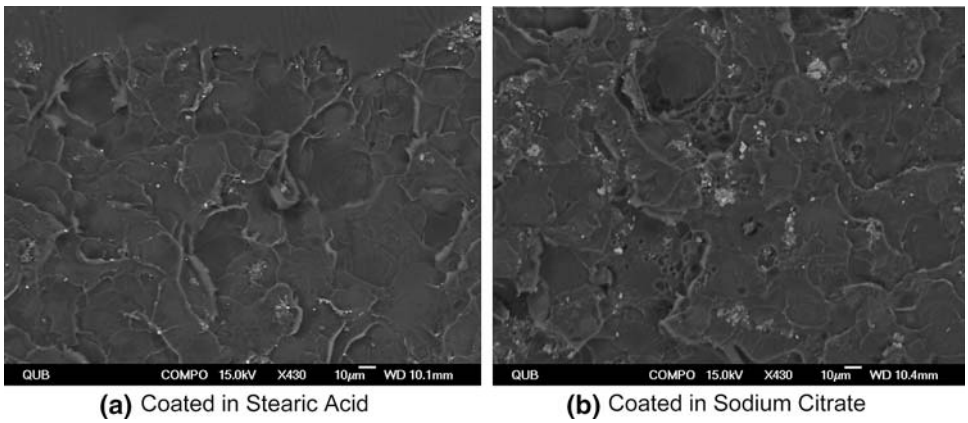

Fig. 1 Backscattered SEM image of coated nanoCaCO$_3$ coated with stearic acid in bone cement.

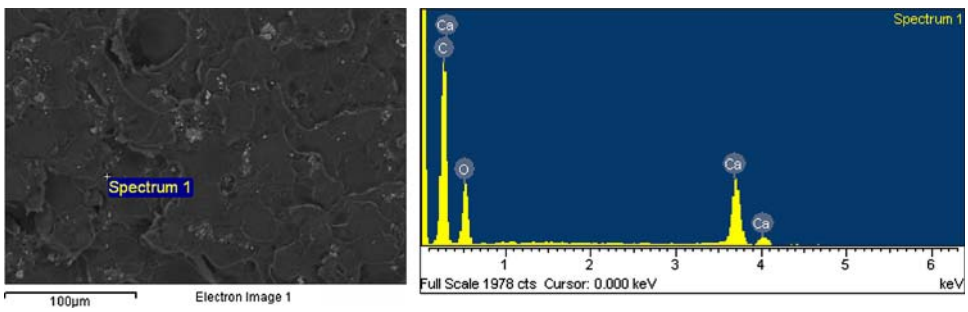

Fig. 2 EDX results confirming the presence of nanoCaCO$_3$ in the bone cement

Table 7 Recommended factor levels obtained from DOE

Factor	Units	Type	Factor level
Percentage nanoclay	% (wt)	Numerical	0.25
Mixing type	–	Categorical	Probe
Type of coating	–	Categorical	SA

When comparing the results of this study to other studies, which have used additives to improve the mechanical properties of acrylic based bone cement [4, 11–18], the results are encouraging in terms of improvements in mechanical properties and merits further investigation. Reports have shown that the use of other nano-scale fillers such as nano$BaSO_4$ as opposed to micro$BaSO_4$ in acrylic bone cement did improve its mechanical properties, in particular the work of fracture, but only to equal that of radiolucent cement [12]. However, results from this study show that incorporation of nano$CaCO_3$ to acrylic bone cement can result in greater mechanical properties when compared to radiolucent cement. The addition of SC coated nanoclays did produce comparable improvements in energy to maximum load [10] when compared to the addition of nano$CaCO_3$, while less improvements were seen with the addition of uncoated nanoclays [9]. Using the recommended factor levels found in this study for the incorporation of nano$CaCO_3$ to acrylic bone cement improves the material's mechanical properties. Not only is nano$CaCO_3$ biocompatible but due to the low percentage loadings that can be added into the cement it will not significantly alter the consistency or workability of the cement. This is not the case for other additives (e.g. metal fibres, aramid fillers or high loadings of nanoclay) that have been previously investigated [4, 30]. Therefore, the application of nano$CaCO_3$ in acrylic bone cement provides the best solution in terms of biocompatibility, workability and improvement in mechanical properties.

5 Conclusions

Nano$CaCO_3$ was added to acrylic bone cement and a DOE approach was used to analyse the factors affecting the mechanical properties. Significant improvements in mechanical properties were achieved on adding less than 1% (wt) nano$CaCO_3$. Since $CaCO_3$ is biocompatible, readily available material, the addition of these particles to acrylic bone cement is a feasible opportunity to improve the mechanical properties of the final cured cement. DOE demonstrated that the best conditions for the addition of nano$CaCO_3$ are 0.25% (wt), which was coated in SA and then incorporated with the liquid monomer constituent of the cement using an ultrasonic probe prior to mixing of the polymer powder and liquid monomer. These conditions provided improvements of approximately 21% energy to maximum load, 10% elastic modulus, 7% bending strength and 8% bending modulus when compared with bone cement containing no $CaCO_3$ nanoparticles. Although cement containing nano$CaCO_3$ coated in SC enhanced the energy to maximum load by 28% and the elastic modulus by 14%, it is not recommended as a factor in the production of nanocomposite bone cement due to the reduction in bending strength and modulus.

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