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Biomechanical evaluation of healing of critical-sized long bone defects in rats treated with biphasic hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) bioceramic scaffolds

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Abstract

Critical-sized bone defects are those that would not heal spontaneously despite surgical stabilisation. These defects are treated using autografts, allografts, xenografts and synthetic bone grafts. The present study was undertaken to evaluate the efficacy of biphasic hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) bioceramic scaffolds in treating critical-sized segmental bone loss in rats. The study was conducted in sixty male Wistar rats aged between 8-12 weeks, weighing 200-250 g body weight with critical-sized defects in the right femur. A six-mm segmental mid-diaphyseal femoral defect was created under general anaesthesia. The bone defect was bridged with biphasic hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) bioceramic scaffolds and retained in position with microplate and screws. Fifteen rats were sacrificed as per the guidelines of CCSEA during the 4th, 8th, 12th and 16th week post-surgery. The treated bone and contralateral femur were harvested and subjected to a three-point bending test, torsion test and compression test to compare the regained strength of the repaired right femur with that of the intact contralateral left femur. From the present study, it was observed that the use of biphasic hydroxyapatite and β-tricalcium phosphate bioceramic scaffolds in the treatment of critical-sized long bone defects in rats resulted in biomechanical properties of the healed right femur greatly superior to the femur with untreated critical-sized diaphyseal defect by sixteenth week post-surgery and was only slightly inferior to the normal intact left femur. The results were suggestive of using biphasic hydroxyapatite and β-tricalcium phosphate bioceramics scaffolds as a safe and promising alternative for the treatment of critical-sized bone defects.

Keywords: *Beta-tricalcium phosphate, bioceramic scaffold, compression test, hydroxyapatite, three-point bending test and torsion test.*

Critical-sized bone defects are those that would not heal spontaneously despite surgical stabilisation (Reichert, 2010). Such fractures often fail to heal by normal processes of bone healing. Hence, such bone injuries require scaffolds that promote the bridging of fracture fragments (De Coster *et al.,* 2004). Hydroxyapatite (HA) ceramics are widely used as bone substitutes due to their osteo-conductivity and bio-compatibility (Cacciotti, 2014). The disadvantage of plain hydroxyapatite was its slow rate of biological interaction (Thian *et al*., 2006). Biphasic calcium phosphate bioceramics composed of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) have ideal properties of synthetic bone graft such

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as tuneable resorption, bioactivity and intrinsic osteo-induction (Rompen *et al.,* 2006). The present study was undertaken to evaluate the biomechanical properties of long bones at varying time points post-implantation of biphasic hydroxyapatite (HA)and β-tricalcium phosphate (β-TCP) bioceramic scaffolds during treatment of critical-sized segmental bone loss in rats. Three-point bending test, torsion test, and compression test are the commonly used tests to evaluate the biomechanical properties of bioceramic scaffolds.

Materials and methods

Sixty adult male Wistar rats with ages between 8-12 weeks weighing 200-250 grams body weight were selected for the study. The experimental animals were procured from the Small Animal Breeding Station (SABS), College of Veterinary and Animal Sciences (CVAS), Mannuthy, Thrissur and maintained as per the guidelines of CCSEA and PCA Act 1960 (Amendment: 1998, 2001 and 2006) (Pandey and Sharma, 2011). Pre-surgical evaluations of all the rats were conducted and surgical creation of critical-sized defects in the right femur preferred femoral diaphysis as per Costa *et al.* (2011). A 6 mm segmental mid-diaphyseal right femoral defect was created under general anaesthesia using injections of xylazine, ketamine and midazolam administered intraperitoneally. The bone defect was bridged with porous cylindrical biphasic hydroxyapatite (HA) and β- tricalcium phosphate (β-TCP) bioceramic scaffolds and retained in position with microplate and screws. Muscle and skin layers were closed using 7-0 nylon in a simple continuous suture pattern. All the rats were maintained on standard feeding and management practices and observed for a period of 16 weeks. Analgesic and antibiotic were administered during the immediate post-operative period. The surgical wounds were dressed using antibiotic spray containing neomycin sulphate, polymyxin B sulphate and bacitracin zinc post-operatively and skin sutures were removed after two weeks. Fifteen rats were sacrificed as per the guidelines of CCSEA during the 4th, 8th, 12th and 16th week (T1, T2, T3 and T4). The treated bone (after removal of microplate and screws) and contralateral femur were harvested and subjected to a three-point bending test, torsion test and compression test and compared between each as per Ekeland *et al.*(1981). The tests were carried out in a Universal Testing Machine 'Instron' as per modifications suggested by Saunders *et al.* (2010). The acquired data was collected and analysed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). Descriptive statistics are given by mean ± standard error of the mean. Group comparisons were performed by ordinary one-way ANOVA and Student's t-test. *P*-values < 0.05 (significance level α = 0.05) were declared as statistically significant different (Prodinger, *et al.*, 2018).

Result and discussion

Three-point bending test

The bending force (N) required to break the normal bone increased from a mean of 23.07±0.30 N at four weeks, to 36.26±1.00 N at eight weeks, to 51.78±0.60 N at twelve weeks and to 68.24±0.22 at sixteen weeks (Fig. 1, 3). The force required to break the normal bone increased every four weeks with the maturation of bone by 36.37 per cent, 29.97 per cent and 24.12 per cent by the 8th, 12th and 16th week respectively. These observations were in accordance with that made by Indrekvam *et al.* (1991), who observed that ultimate bending load to failure in rat femur, increased steadily over a 52 week study period. This higher capacity was attributed to increased

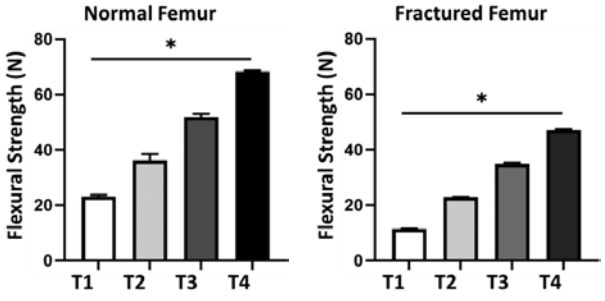

Fig.1. Change in flexural strength of normal femur and fractured femur with age

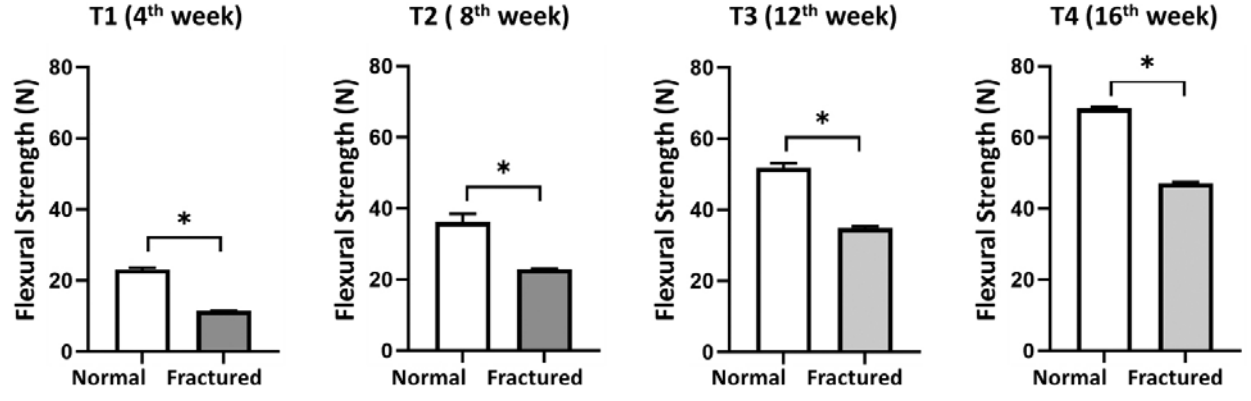

Fig. 2. Comparison between flexural strength of normal femur and fractured femur at 4th, 8th, 12th and 16th week post biomaterial implantation

dimensions of the bone with age rather than its material properties. Ekeland *et al.* (1981) observed an increase in bending and torsional strength from young age till 14 weeks of age.

The bending force (N)required to break the healing bone increased from a mean of 11.43±0.07 N at four weeks, to 22.92±0.09 N at eight weeks, to 34.92±0.23 N at twelve weeks and to 47.08±0.17 N at sixteen weeks (Fig. 1, 3). In all the cases, the bones were fractured at the region of the segmental defect. The force required to break the fractured bone increased every four weeks with the maturation of bone by 50.13 per cent, 34.36 per cent and 25.82 per cent by the 8th, 12th and 16th week respectively. A part of this result was found to be in accordance with that made by Wheeler *et al.*(2000), who reported that the maximum bending loads for the rat femur at the fourth and sixth were notably lower than that of the eighth week.

The tests revealed that the healing bone had regained 49.54 per cent of flexural strength of the normal bone by the fourth week, which subsequently increased to 63.20 per cent, 67.43 per cent, and 68.99 per cent by the 8th, 12th and 16th week post-surgery respectively against observations in similar periods (Fig. 2). This observation was not in agreement with the reports of Ekeland *et al.* (1981) who observed that normal strength and bending values were restored in fractured (non-segmental) femur by eight weeks. This observation was also not in accordance with that made by Meyer *et al.* (2001) in rats, who observed that normal biomechanical values were restored in fractured (non-segmental) femur by 12 weeks. Observations made by Dinesh (2018) using HASi scaffold as femoral bone replacement material showed slightly higher flexural force to failure but comparable to the present observation.

Torsion test

The torsional force (N) required to break the normal bone increased from a mean of 19.42±0.08 N at four weeks, to 21.59±0.14 N at eight weeks, to 23.76±0.08 N at twelve weeks and to 25.51±0.18 N at sixteen weeks (Fig. 4, 6). The force required to break the normal bone increased every four weeks with the maturation of bone by 10.05 per cent, 9.13 per cent, and 6.86 per cent by the 8th, 12th and 16th week respectively. These observations were in accordance with that made by Indrekvam *et al.* (1991) who observed that ultimate torsional load to failure in rat femur, increased steadily over a 52 week study period. This higher capacity was attributed to the increased dimensions of the bone rather than its material properties. Ekeland *et al.* (1982) observed a steady increase in torsional strength from young age till 14 weeks of age.

The torsional force (N) required to break the healing bone increased from a mean of8.52±0.09 N at four weeks, to 11.43±0.14 N at eight weeks, to 14.48±0.12 N at twelve weeks and to17.74±0.05 N at sixteen weeks (Fig. 4, 6). In all the cases, bones were fractured at the region of the segmental defect. The force required to break the fractured bone increased every four weeks with the maturation of bone by 25.45 per cent, 21.06 per cent, and 18.37 per cent by the 8th, 12th and 16th week respectively. Dinesh (2018), while working on the healing of non-segmental bone defect of rat femur, reported a significant increase in maximum bending load, bending rigidity and fracture energy between 6 and 12 weeks post-fracture. This observation was also found in accordance with that made by Ekeland *et al.* (1981), who observed increased resistance to torsional moments in the femur with an increase in the age and weight of animals, in the early phases of non-segmental fracture repair. Observations made by Dinesh (2018) for torsional strength to failure of the femur using the HASi scaffold were found to be higher but comparable to that using the HA-βTCP scaffold.

The test revealed that the healing bone had regained 43.87 per cent torsional strength of the bone by the fourth week, which subsequently increased to 52.94 per cent, 60.94 per cent and 69.54 per cent by the 8th, 12th and 16th week post-surgery respectively against observations in similar periods (Fig. 5). Ekeland *et al.* (1981) observed that normal strength and torsion values were restored in fractured (non-segmental) rat femurs by 13 weeks.

Compression test

The compressive force (N) required to break the normal bone increased from a mean of 42.72±0.06 N at four weeks, to 45.46±0.12 N at eight weeks, to 48.67±0.11 N at twelve weeks and to 52.35±0.16 N at sixteen weeks (Fig. 7, 9). The force required to break the normal bone

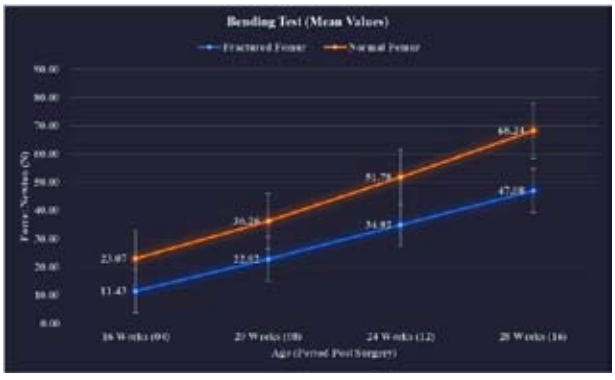

Fig. 3. Comparison between flexural strength of normal femur and fractured femur

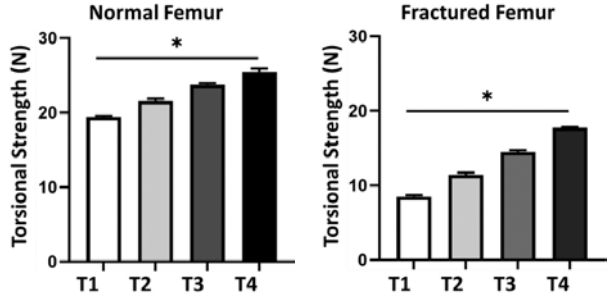

Fig.4. Change in torsional strength of normal femur and fractured femur with age

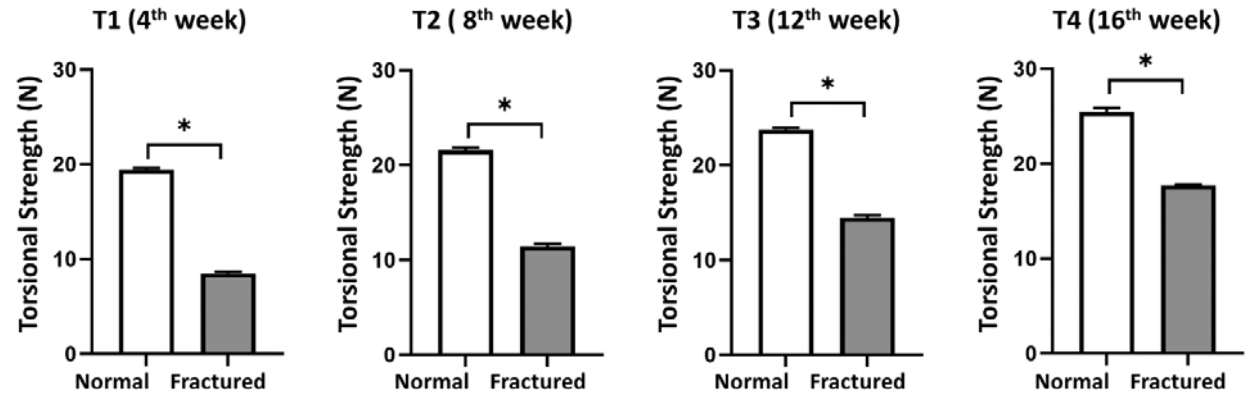

Fig. 5. Comparison between torsional strength of normal femur and fractured femur at 4th, 8th, 12th and 16th week post biomaterial implantation

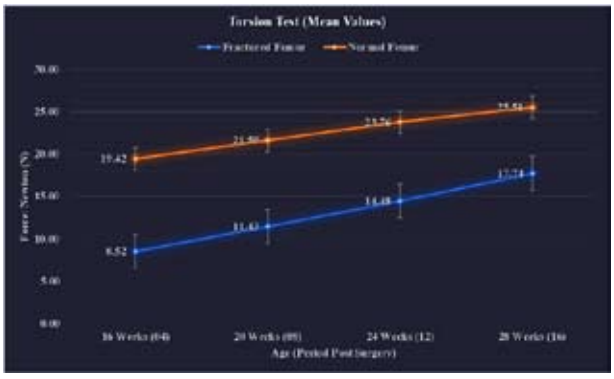

Fig. 6. Comparison between torsional strength of normal femur and fractured femur

increased every four weeks with the maturation of bone by 6.03 per cent, 6.60 per cent, and 7.03 per cent by the 8th, 12th and 16th week respectively. These observations were in accordance with that made by Indrekvam *et al.* (1991), who observed that ultimate compressive load to failure in rat femur, increased steadily over a 52 week study period. This increased resistance was attributed to increased dimensions of the bone rather than its material properties.

The compressive force (N) required to break the healing bone increased from a mean of28.92±0.23 N at four weeks, to 33.17±0.28 N at eight weeks, to 36.99±0.27 N at twelve weeks and to41.37±0.18 N at sixteen weeks (Fig. 7, 9). In all the cases, bones were fractured at the region of the segmental defect. The force required to break the fractured bone increased every four weeks with the maturation of bone by 12.81 per cent, 10.33 per cent, and 10.59 per cent by the 8th, 12th and 16th week respectively. A work done by Kirker-Head *et al.* (2007) using BMP-silk composite as femoral replacement material showed compressive force to failure far less than our present observations, indicating better healing strength by HA-βTCP bioceramic scaffolds. The compressive strength to failure of the femur using the HA-βTCP bioceramic scaffold was found to be less but comparable to that using the HASi scaffold in comparison with work done by Dinesh (2018).

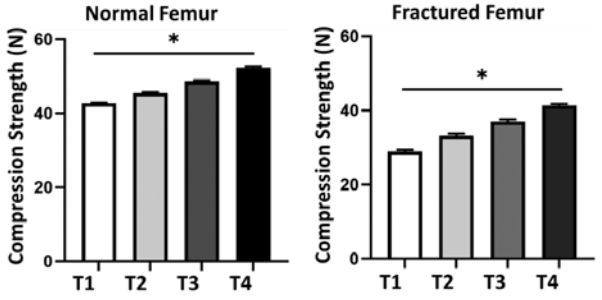

Fig. 7. Change in compression strength of normal femur and fractured femur with age

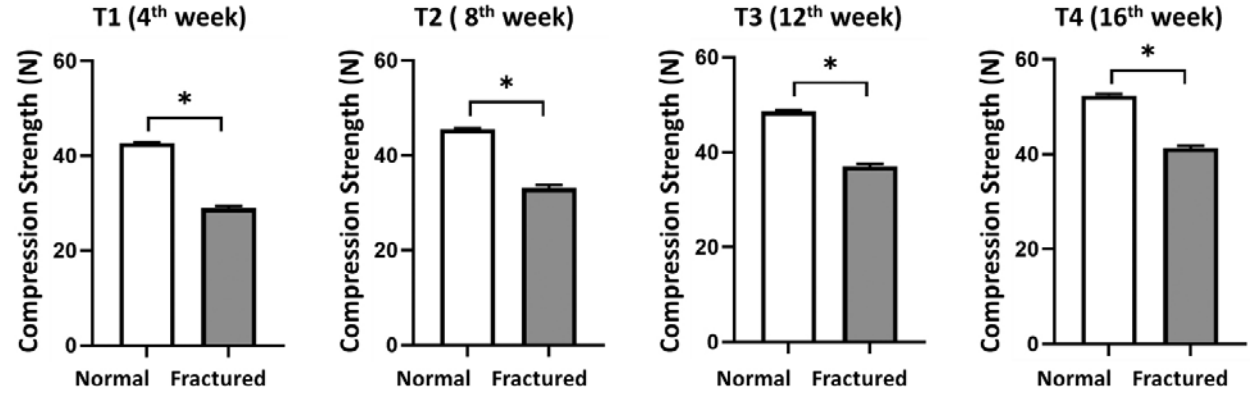

Fig. 8. Comparison between compression strength of normal femur and fractured femur at 4th, 8th, 12th and 16th week post biomaterial implantation

Biomechanical tests revealed that the healing bones regained 67.69 per cent compressive strength of the untreated bone by the fourth week, which subsequently increased to 72.95 per cent, 76.48 per cent and 79.02 per cent by the 8th, 12th and 16th week post-surgery respectively against observations in similar periods (Fig. 8). Notomi *et al.* (2000) suggested that the increase in strength of the healed femur was a result of an increase in periosteal bone formation and reduction of endocortical mineral apposition.

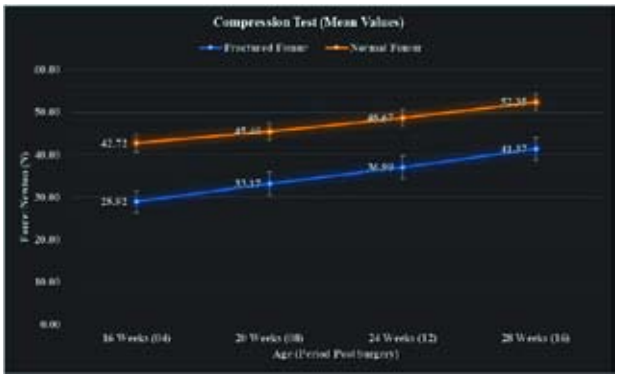

Fig. 9. Comparison between compression strength of normal femur and fractured femur

Conclusion

Biomechanical evaluation of healing of critical-sized femur defects in rats were conducted using a three-point bending test, torsion test and compression test and were found useful for the evaluation of flexural, torsional and compressive mechanical properties of healing bone with diaphyseal critical defect treated with HA and β-TCP bioceramic scaffolds. This study revealed that the biomechanical properties of the femur treated with bioceramics were greatly superior to the femur with untreated critical-sized diaphyseal defect (which does not heal) and was slightly inferior to the normal intact femur by the sixteenth week post-surgery. The results were suggestive of using biphasic hydroxyapatite and β-tricalcium phosphate bioceramic scaffolds as a safe and promising alternative for the treatment of critical-sized bone defects.

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Conflict of interest

We have no conflicts of interest to disclose.

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