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Comparative evaluation of anatomical vs non-anatomical bone excavation in rabbit long bone defect model

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Abstract
Successful sample collection is an obligatory step in the biomedical research. It is well known that choosing right pre-processing sample collection techniques can improve effectiveness of result to a far extent. We experimentally compared two techniques of sample collection namely group A: Non-anatomical de-muscling and group B: Anatomical dissective de-muscling. Each group comprising of 10 animals undergone critical size (15mm) defects (CSD) in the radius, forming a defective long bone rabbit model. We found that a technique employing an anatomical dissective sample collection has faster sample excavation time without much requirement for post-excavation de-muscling. Based on the results obtained, this study concludes that the anatomical dissective approach of sample collection is a better collection method comparing with that of non-anatomical dissective sample collection. Other comparative diagnostic and biomechanical testing is the future arena of research on sample collection and evaluation.

Keywords: Sample collection, bone, radius, long bone defect, animal model

Introduction
The evaluation of tissue constructs in preclinical level is an inevitable process in the field of biological tissue engineering. *In vitro* and *in vivo* testing have been routinely performed ontissue constructs or substitutes developed through tissue engineering techniques. The major drawback of *in vitro* testing is the high cost involved in the procedure. On flip side, the results obtained from the *in vivo* preclinical testing should be reliable and reproducible considering the ethical, scientific, as well as economic aspects. There are various animal models used in biological tissue engineering and regeneration. Bone tissue engineering is an area where rabbits are widely used as an animal model for pre-clinical studies. Further the results may vary with species, breed, gender, age and defect size and its location (Zeiter *et al*., 2020) [1]. Rabbits are largest animal among the small laboratory animals. The implant associated changes are well distinguished and promptly evidenced in rabbits than in dogs (Mapara *et al*., 2012) [2]. Mostly the researchers' focus towards an innovative and combinatorial tissue engineering strategies and are limitedly sensitized to animal variability and its environment so as to produce a reliable and reproducible results. Presently sample excavation in bone tissue engineering has not been much sensitized among researchers. There is a lack of standard guidelines in this regard. Though sample is the gem of research for further analysis, it is usually sidelined by the inefficient way of collection. The authors hypothesized that anatomical vivisection of bone is faster and efficient way than butchering technique of bone tissue excavation.

Materials and Methods
The study has been conducted in 20 healthy adult rabbits of either sex. The study had approval from IAEC, ICAR-IVRI, India. In all the rabbits, right forelimb 1.5 cm radial defects were created in both groups (group A & group B). Briefly, after xylazine (6mg/kg body weight I/M) and ketamine (60 mg/kg boy weight I/M) administration, with the aid of oscillating saw, right radial 1.5cm mid diaphysis critical size defects were created. The rabbits were maintained in individual cages up to 90th postoperative day under 12hr: 12hr day and dark light cycle at about 28^{0}C in an institute lab animal shed. After 90th day, the rabbits were over-anaesthetized with 5% thiopental sodium@70 mg per kg via intra-cardiac route.

The rabbits were divided randomly into two groups consist of ten (10) animals each: group A (Butchering bone tissue excavation) and group B (Anatomical bone tissue excavation).

Materials in excavation

Water, no.21 Bard parker blade with handle, scissor, thumb forceps, weighing balance, surgical drape (Green colour), blotting paper, polythene disposable spread sheet, hand held HD mobile camera (64MP).

Methods of bone tissue excavation

The animals of both the group were casted on left lateral recumbency. Upon the right forelimb, wet water solution was sprayed prior to the start of excavation. Out of 20 animals half of the animals undergone de-muscling via butchering approach of radius-ulna excavation and remaining half undergone de-muscles via anatomical vivisection approach post-humously. The excavation time (starting from time of skin incision to complete removal of bone from animal body), number of muscle fragments (number of muscle pieces), weight of the muscle (muscle fragments), bone damage scoring (1 for no damage, 2 damage restricted to bone other than critical size defective site of bone, 3 for damage of critical size defective site bone) and gross observation (scoring by study blindfolded researcher 1 for fit for further analysis, 2 for fit after further de-muscling 3 unfit for further analysis due to damage of tissue interest site-here we considered critical size defect as our interest site) were the parameters examined in this study. The surgery done by blindfolded researcher (who is institute experimental animal operative surgeon). The surgery has done first non-anatomical excavation and gap of after 3 months anatomical dissective excavation carried, to minimize experience and dexterity bias.

The detailed approach as follows

Butchering approach of bone tissue excavation

In this approach the skin has been incised cranio-laterally and the muscles were scrapped around radius and ulna by using number 21 Bard-parker blade. Separated and severed wherever muscle mass was felt to cut. Then both proximal and distal joint of forearm region was dislocated to remove the radius and ulna bones. The final excavated bone tissue of non-anatomical approach showed in fig. no 2.

Anatomical vivisection approach of bone tissue excavation

As per butchering approach skin has been excised cranio-laterally and further this approach as follows

The severance of attachment of epitrochlearis, long head of triceps brachii lateral head of triceps brachii and dorsal lifting of deltoideus and associated muscles of proximal portion of radius-ulna.

Then passing of no 21 bard parker blade around the bone of forearm underneath ulna and driving over and above of critical size defect site of radius. Then By holding radius ulna in hand, disarticulation of radio-ulnar-carpal joint by severing in flexed position of the joint (Mukhopadhyay *et al*., 2020) [3]. The anatomical vivisection of radius CSD model showed in figure no 1a to fig 1k.

Statistical analysis

Parametric with normal distributive data and nonparametric data analyzed with independent student t test and Mann-Whitney U test respectively by using SPSS 16.0.

Results and Discussion

Table 1: Result of comparative parameters of the study of bone tissue excavation

Parameter	Non-anatomical dissection	Anatomical dissection	p-value	t value	Degree of freedom
Rabbit body weight (Mean±SD)	2.4675±0.52730	2.6050±0.46223	0.588 (>0.05)	-.555	18
Excavation time (Mean±SD)	6.2150±0.81138*	4.6000±0.39551	0.000 (<0.05)	5.061	18
Number of muscle pieces(Mean±SD)	5.1250±1.45774	6.5000±1.19523	0.058(>0.05)	-2.063	18
Weight of muscle(Mean±SD)	14.2500±2.60494*	11.2500±1.66905	0.016(<0.05)	2.743	18
Bone weight(Mean±SD)	3.3750±0.51755	3.0625±0.41726	0.205(>0.05)	1.330	18
Scores	Non-anatomical dissection	Anatomical dissection	p-value	U value	
Roentgenogram (pre)	1(0)	1(0)	0.960 (p>0.05)	32.0	
Bone damage score Median(IQR)	2(1.5)	1(0.5)	0.223 (p>0.05)	22.0	
Gross observation score Median(IQR)	2(0.5)	1(0.5)	0.0238(p<0.05)	10.0	

SD: Stanadard Deviation; IQR: Interquartile Range; *significant (*p*-value<0.05)

The collection of sample is paramount importance of good outcome of results. In this study result has been depicted in table no.1

Rabbit body weight and roentgenogram

There is no significant difference in rabbit body weight between the anatomical and non-anatomical technique of bone sample collection. Perhaps preoperative roentgenogram (Computed radiography: Toshiba X ray machine; CR reader: REGIUS ΣII, Konica minolta) radiography of all the animals in the study confirmed that there were no bone pathological changes which may downgrade the result of study.

The excavation time

Sample removal time was significantly longer in non-anatomical dissection than anatomical dissection method. This may be attributed by selective dissection and easy separation aided by severance at insertion and origin points of muscle attachment. In non-anatomical method lack of strict adherence of procedure and excavation *en masse* would be the reason.

Number of muscle fragments (number of muscle pieces)

The number of muscle pieces of sample excavation between the two groups had not shown any significant differences. But size of muscles varies grossly. This could be attributed by the result of muscle weight difference between the groups.

Weight of the muscles

Weight of the muscles between two groups had significant remarks. This is due to removal of muscle in non-anatomical governed more muscles de-muscled from body prior to disarticulation. Hence these non-anatomical had increased muscle weight than anatomical dissection approach. Anatomical dissection approach initially cut from epitrochlearis reduces the muscle mass at the time of disarticulation of humero-radial joint. Contrary non-anatomical yields more muscle fragments while separating muscles around humero-radial joint.

Bone weight

Bone weight after de-muscling shown no significant difference between the groups. This could be due to animals of both group has same age, nutrition, managerial and operative conditions. The critical size model also has been created as alike. Hence post excavation weight of mass of bone has no significant changes.

Bone damage score

The result of bone damage scoring after de-muscling of bone represents there is no significant difference between the groups. Although no significant difference statistically, it has been noticed by author that the two samples of non-anatomical dissection approach has edge over damage than anatomical dissection of sample removal.

Gross observation score

Gross observation of bone score in regards with goodness of fit for future sample analysis result showed that there is a significant difference between the groups. This represents non-anatomical dissection of sample collection approach is needed further meticulous de-muscling prior to sample storage or submission for further analysis.

Gross observation score luminescence the sample interest and muscle, on contrast bone weight in both the group not significantly varied. This could be attributed due to difficulty in de-muscling and inaccessible site of muscle attachment in non-anatomical approach, thereby longer sample excavation time.

Fig 1a: Antero-lateral skin incision
Fig 1b: Separation of forearm skin
Fig 1c: Severance of epitrochlearis
Fig 1d: Severance of lateral head of triceps brachii
Fig 1e: Severance of long head of triceps brachii, deltoideus and its associated muscles
Fig 1f: Severance of extensor attachment from bone by passing blade underneath the extensors
Fig 1g: Severance of flexors (flexor carpi radialis and flexor digitorum profundus from bony contact by passing blade around the bone of forearm
Fig 1h: Severing of all forearm muscle attachment at its proximal or distal portion close to joint
Fig 1i: Disarticulation of humero-radial joint
Fig 1j: Disarticulation of radio-ulnar vis a vis carpals
Fig 1k: Bone and separated muscles after anatomical excavation
Fig 2: Bone and separated muscles after non-anatomical excavation

Conclusion

Anatomical dissection has advantage over non-anatomical method of sample collection due to short sample collection time and further de-muscling required is very minimal. The small sample size and lack of Roentgenogram comparison between the groups after sample collection are the limitation of this study. Hence future study comparison of sample collection based on other diagnostic imaging and biochemical testing improves reliability of such studies.

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