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Evaluation of DNA Content of Harvested Bone Prior to and After Cryopreservation at - 80º C for 6 Months in Bone Bank

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Abstract: ***This study investigates the impact of cryopreservation at - 80C for six months on the DNA content of bone grafts, which is crucial for the success of orthopedic surgeries. Conducted at UCMS GTB Hospital, Delhi, bone samples from four patients were analyzed using Feulgen staining before and after the cryopreservation period. The results showed a complete absence of DNA content in all samples post - cryopreservation, highlighting a significant impact on the cellular viability of the grafts. This absence may affect the osteogenic potential and overall success of bone grafts. These findings underscore the need for further research to improve preservation techniques and enhance the functionality of bone grafts in clinical applications.***

Keywords: Bone grafts, Cryopreservation, DNA content, Orthopedic surgery, Osteogenic potential

1. Introduction

Successful graft incorporation is defined as the ability of the transplanted tissue to work like the original tissue; that is, to maintain its mechanical integrity and function during and after the process of incorporation. A significant number of orthopaedic procedures involve disruption or injury of bone at the surgical site. To assist in the bone healing response during and/or after the surgery, most surgeons incorporate bone - grafting products (1).

Necessarily, the use of bone transplants in orthopaedic procedures has become crucial to treat a great number of bone diseases (1). Surgeons take into consideration the type of procedure, comorbidities, and the expected osteogenic capacity of the patient to determine the types of bone grafting products that are needed (2). There are various materials available to use in bone grafting, ranging from autologous bone (autografts), allograft, or xenograft (grafts from a non - human origin) (3).

Autografts remain the gold standard, but its use is complicated by limited availability and morbidity to the donor site; thus, the use of allograft is often required. Allograft, however, still pose some possible complications, including the transmission of infectious disease and immunogenic response (4). It has been shown that the main difference between autologous bone and all types of allograft is the lack of viable donor cells that can contribute to healing, and the potential for immunological reactions (5 - 9). Considering the high demand for bone grafts, many ways of processing and storage bone tissue for clinical application have been proposed and used in Tissue Banks around the world. Among them, the deep - frozen (- 80 degree C) is the most widely used and accepted method. The ultralow freezing temperature is reached in freezers that go as low as - 80 degree C with graphical systems constantly monitoring the temperature, having their own power generators and emergency alarms alerting when temperature increases (6 - 15). The rationale for conducting a study on the "Evaluation of DNA Content of Harvested Bone Prior to and After Cryopreservation at - 80°C for 6 Months in Bone Bank" is grounded in the critical role that bone grafts play in orthopaedic surgeries and the widespread use of cryopreservation in bone banks to store these grafts. Bone grafts are essential for a variety of reconstructive surgeries, and their success is significantly determined by the viability and osteogenic potential of the graft which is, in turn, influenced by the viability of the cellular components, including DNA.

Cryopreservation at - 80°C is a common method for storing bone grafts as it is believed to maintain the structural integrity and potential biological activity of the bone. However, the actual impact of such ultra - low temperatures on the DNA content of bone cells, which is crucial for cell replication, differentiation, and overall graft success, is not fully understood. DNA integrity is a marker of cell viability and has implications for the osteogenic potential of the graft. If DNA degrades or is significantly altered during storage, the graft might not integrate well or support new bone formation upon transplantation.

Furthermore, understanding DNA viability post - cryopreservation has broader implications for the fields of tissue engineering and regenerative medicine. It can inform better storage protocols, improve graft success rates, and potentially reduce the risk of graft failure. It also contributes to the ongoing discourse on the best practices for bone banking, which is essential for making allografts readily available for surgeries. Therefore, this study is aimed at filling the knowledge gap regarding the impact of long - term cryopreservation on DNA integrity in bone cells. By evaluating the DNA content before and after cryopreservation, the study seeks to provide empirical evidence that can lead to improved bone graft preservation techniques, ultimately enhancing patient outcomes in orthopaedic surgeries. This study aims to assess the DNA content of the harvested bone histologically using Feulgen stain prior to and after cryopreservation at - 80°C for 6 months.

2. Methods

This hospital - based study was meticulously designed and conducted in the Department of Orthopaedics and Department of Pathology, UCMS & GTB Hospital, Delhi.

The primary objective was to evaluate the DNA content in harvested bone allografts before and after a 6 - month period of cryopreservation at - 80°C. Over one and a half years, bone samples were surgically obtained from patients who voluntarily agreed to donate their bone for this research. Initially, a rigorous patient selection process was implemented. Potential donors were screened based on a predefined set of criteria for bone procurement in the bone bank. These criteria were set to ensure the quality and safety of the bone grafts, as well as the suitability and health of the donors. Only patients who met all the requirements were included in the study. Despite efforts to recruit a larger sample, only four patients were ultimately enrolled due to various constraints including the readiness and consent of the patients, as well as time limitations. Once patients consented to participate, they underwent a detailed clinical examination. Written informed consent was obtained from each, ensuring they were fully aware of the study's nature, purpose, and potential implications. This process underscored the voluntary and deliberate involvement of each subject, adhering to ethical standards. The harvested bone samples underwent a standard procedure for cryopreservation, storing them at - 80°C for six months. After this period, they were retrieved for analysis. The DNA content of the samples was then assessed histologically using Feulgen stain both before and after the cryopreservation process. For data management and statistical analysis, the study utilized SPSS (Statistical Package for the Social Sciences) version 24.0 for Windows. This robust software enabled the research team to perform complex statistical analyses, ensuring accurate and reliable results. Throughout the study, ethical guidelines were strictly followed. The Institutional Ethical Committee granted approval after a thorough review of the study aims and methodologies. This ethical oversight ensured that all research activities were conducted responsibly, upholding the dignity and rights of the participants while aiming to contribute valuable knowledge to the field of orthopaedic medicine.

3. Result

The age wise distribution of patients showed that the age range was 50 - 74 years. The mean age of the patients was 62.25 + 10.01 years (Table 1 and Table 2). Only one patient was male (25% of the entire sample and rest 03 patients were females (75% of the entire sample).

Table 1: Age and gender description of study patients

S. No.	Histopathology Number	Age	Gender
1	2305/ 20	60	Male
2	2303/ 20	74	Female
3	2301/ 20	50	Female
4	2299/ 20	65	Female

Table 2: Age distribution of study participants

	N	Mean	SD	Minimum	Maximum
Age	5	62.25	10.01	50	74

Table 3 shows the comparison of bone matrix findings at the time of harvest and beyond 06 months of cryopreservation. Factors that were assessed are detailed in Table 3.

Table 3: Comparison of Bone Matrix findings at time of harvest and beyond 06 months of cryopreservation

	At initial harvest		Beyond 06 months of harvest		P- Value
	No. of samples taken	Bone Matrix findings	No. of samples taken	Bone Matrix findings	
Inorganic Matrix	4	Present	4	Present	1,000 (NS)
Organic Matrix	4	Present	4	Present	1,000 (NS)
Lacunated Architecture	4	Present	4	Present	1,000 (NS)
Other Cells	4	Present	4	Present	1,000 (NS)
Feulgen Stain (DNA content)	4	Present	4	Absent	1,000 (NS)

NS: Not significant

It was observed that the findings were similar at the time of harvesting and beyond 06 months of cryopreservation. However, only the DNA content (assessed by Feulgen staining) was absent in all the assessed samples beyond 06 months of cryopreservation. None of the parameters were significantly associated ($p > 0.05$).

Table 4: Number of Osteocytes in bone matrix at the time of harvest

Histopath Number (ID)	At the time of harvesting	Beyond 06 months of cryopreservation
	Number of Osteocytes	Number of Osteocytes
2305/ 20	10	15
2303/ 20	40	11
2301/ 20	13	20
2299/ 20	20	21
Mean $\pm$ SD	Mean= 20.75 + 13.50 (SD)	Mean= 16.75 + 4.646 (SD)

Table 4 shows the comparison of number of osteocytes present in the Bone Matrix at the time of harvesting and beyond 06 months of cryopreservation. A change was observed beyond 06 months of cryopreservation. An increase in the number of osteocytes in some slides beyond 06 months of cryopreservation has been observed, however, it can be regarded that the field of focus may have been changed in the microscope in both the examinations. Hence, the statistical analysis of this aspect is not conducted and no p - values has been obtained in this regard. There was a change in the number of osteocytes, and the mean value has declined after 06 months of cryopreservation, but the finding cannot be attributed to statistical evaluation for determining a significant outcome.

4. Discussion

The results of the study indicated an age range of patients from 50 - 74 years, with a mean age of 62.25 ± 10.01 years, predominantly female. In evaluating the bone matrix, it was found that while most parameters remained consistent before and after a 6 - month period of cryopreservation, the DNA content was completely absent in all samples post - cryopreservation. This absence signifies a notable impact of

prolonged ultra - low temperature storage on cellular components. Additionally, a variable pattern was observed in the number of osteocytes present in the bone matrix post - cryopreservation; some samples showed an increase, possibly attributable to differing microscopic focus during evaluation. However, due to the lack of significant statistical association and the inability to obtain p - values for this parameter, these findings are inconclusive and highlight the need for further investigation into the impact of cryopreservation on osteocyte viability.

The component assessed was the DNA content of viable cells using Feulgen staining. In the present research, none of the survived cells indicated any presence of DNA content. To our knowledge, there is no such study in the existing literature, which recognizes the change in DNA content post - deep freezing. However, one study demonstrates that the processing involved in preparing fresh deep frozen bone grafts preserves blood structure and donor cells, including nuclear material (10). Their study showed that the cells derived from frozen grafts were morphologically indistinguishable from those grown out of freshly harvested trabecular bone, and had a similar mRNA profile with respect to osteoblast - related genes. Also folsch et al reported that Short - term storage up to 6 months is recommended with −20 °C and −40 °C for a longer period (AATB), and EATB recommends storage at −40 °C and even −80 °C while the regulations of the German German Medical Association (Bundesärztekammer) from 2001 recommend storage at −70 °C. Duration of storage at −20 °C can be maintained at least for 2 years. The potential risk of proteolysis with higher storage temperatures remains, but a definite impairment of bone ingrowth due to a storage at −20 °C was not shown in clinical use, and no adverse biomechanical effects of storage at −20 °C could be proven. Biomechanical studies showed no clinically relevant impairment of biomechanical properties of cancellous bone due to different storage temperatures. Sterilization procedures bear the advantage of inactivating enzymatic activity though reducing the risk of proteolysis. In those cases a storage temperature of −20 °C can be recommended for at least a period of 2 years, and the risk of undesired effects seems to be low for native unprocessed bone. (11)

5. Conclusion

In conclusion, the study aimed to evaluate the DNA content in harvested bone samples before and after cryopreservation at - 80°C for 6 months. The results indicated a complete absence of DNA content in all samples post - cryopreservation. This finding suggests that the preserved cells do not retain viable DNA after being stored at ultra - low temperatures for this duration, a factor that might affect the osteogenic potential and the overall success of bone grafts. While the study contributes to our understanding of the impact of cryopreservation on bone grafts, the clinical implications of the loss of DNA content in bone grafts need further investigation. It also underscores the importance of considering storage conditions and duration in tissue banking to optimize graft viability and functionality. Further research is needed to explore alternative preservation methods or modifications to current protocols to enhance the preservation of DNA and other cellular components critical for successful bone grafting.

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