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Comparative Evaluation Of Mineral & Crystalline Pattern Of Dfdba & Differnt Cbr Scaffolds

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ABSTRACT
The outcomes of phase I periodontal treatment are confined to the reduction of inflammatory responses and the restoration of soft tissue; however, this phase does not achieve the restoration and regeneration of the hard tissue components of the periodontium. Thus we need special techniques like use of bone grafts to plays crucial post-operative postive role in this treatment. Thus in this study we have used XRD & EDS mapping to assess the difference of DFDBA, SMCC & MCS in their mineral content and crystalline pattern. Where, DFDBA was 0.7×0.7 cm, SMCC was 0.8×0.9 cm and MCS was 0.5×0.5 cm dimention respectively. Upon comparing the surfaces of DFDBA, SMCC, and MCS revealed significant differences in their roughness, porosity, mineral content, and CA/P ratio. Thus, to conclude our research, we can say that, although SMCC demonstrates enhanced bioactivity, integration attributed to its Ca : P & open porosity, yet DFDBA remains a viable option for scenarios that require enhanced porosity & osteoconductivity.

Keywords: *XRD , EDS mapping, CA/P ratio, Roughness, Porosity, Mineral Content, DFDBA, SMCC, MCS , Crystalline Pattern*

1. INTRODUCTION

Periodontal regeneration can be achieved using special techniques, like bone grafts during periodontal surgery and GTR operations, which are mainly meant to restore and regenerate.The mineral content present in the bone grafts like DFDBA & different collagen based regenerative material which had shown ability to reconstruct intraocceous, furcation defects and regenerate PDL & cementum.[1,2] Additionally, it contains immuno-reactive bone morphogenetic protein as well as other physiologically active chemicals that are present in their structure and contribute to the regeneration process.[3,4] Additionally, according to a study, newly developed collagen–hydroxyapatite is accessible in various shapes that may rectify the fault.These shapes also give excellent trimming and handling properties.[5] Recent technological breakthroughs have resulted in the creation of materials that combine collagen that has been injected with hydroxyapatite crystals and β-tricalcium phosphate crystals into their composition. It has been hypothesized that these materials possess increased regenerative characteristics in comparison to collagen membranes on their own.[6] This evaluation of the crystalline pattern and mineral composition of regenerative materials is accomplished via the use of a variety of methodologies. One of them is called X-

ray diffraction (XRD), and the other is called electron dispersion spectroscopy (EDS) mapping.[7] The approach is based on the concept of constructive interference of monochromatic X-rays with a crystalline sample, which enables it to provide insights into the mineral composition as well as crystal patterns.[7] One of the most important factors that determines whether or not a project will be successful is the ratio of inorganic to organic regenerative materials that is obtained from this research.[7]

Current literature indicates a lack of in vitro studies that compare and evaluate the mineral composition and crystalline patterns of demineralized freeze-dried bone allograft (DFDBA) with various CBR bone materials using XRD methodology. Thus an attempt was made to evaluate the differences between the two variables to distinguish the regenerative capacities associated with each.

2. MATERIAL AND METHOD

Experimental design: A total of 5 samples of each material (DFDBA , SMCC & MCS) was analysed, ensuring an adequate sample size for statistical evaluation and comparison.

Samples procurement & preparation : In collaboration with the XRD a detailed analysis of DFDBA with 0.7×0.7 cm, SMCC with 0.8×0.9 cm and MCS with 0.5×0.5 cm dimention was done after drying & powedered with dentin grinder in fine powder particles. Later, samples were stored in sterile bone graft containers.

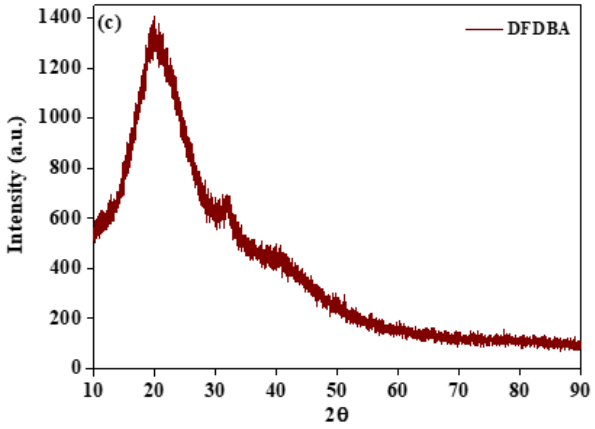
Laboratory Assy analysis: During XRD sample preparation, samples were kept on zero background (silicon) sample holder. Butter paper was used for spreading the sample on sample holder. Further, samples was subjected to XRD under x-ray diffractometer. Ethanol was used for cleaning the sample holder before starting with next sample. Process was repeated till all the samples underwent XRD analysis. EDS mapping for mineral composition on same samples was done. Readings and graphs, on X-axis- Angle (2ɵ) and on Y-axis Intensity was recorded and then analyzed using different software like OriginLab. Furthermore, difference in crystallinity percentage, particle size was noted & tabulated.

3. RESULTS

I. CRYSTALLINE PATTERN

XRD analysis of DFDBA :-

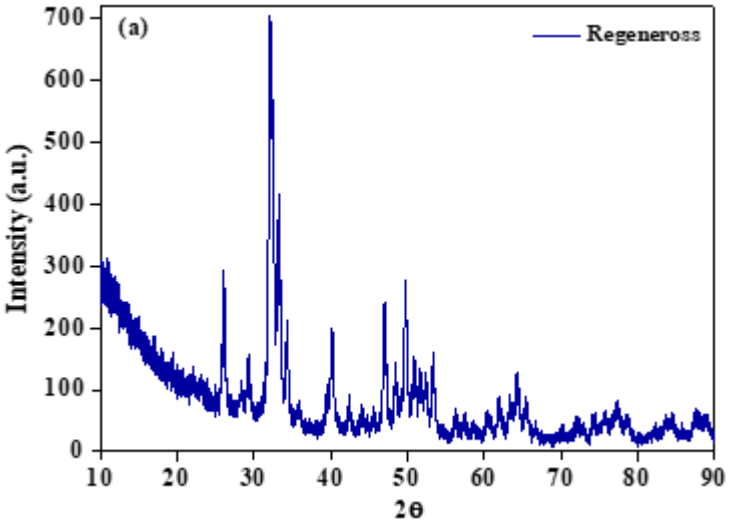
For the XRD analysis, DFDBA was powdered & mounted on zero background silicon sample holder & exposed to X-rays in XRD. Results was expressed in graph pattern plotting Angle (2θ) Versus Intensity (I). Graph having peaks and troughs. For DFDBA, at 19˚ (2θ) with intensity (I) of 1404 & 31˚(2θ) with I of 692; highest peak was observed and this peak was broader as shown in graph 1. The broader peak indicating higher lattice strain within the material & more disordered or amorphous structure.



GRAPH 1: XRD ANALYSIS OF DFDBA

XRD analysis of SMCC :-

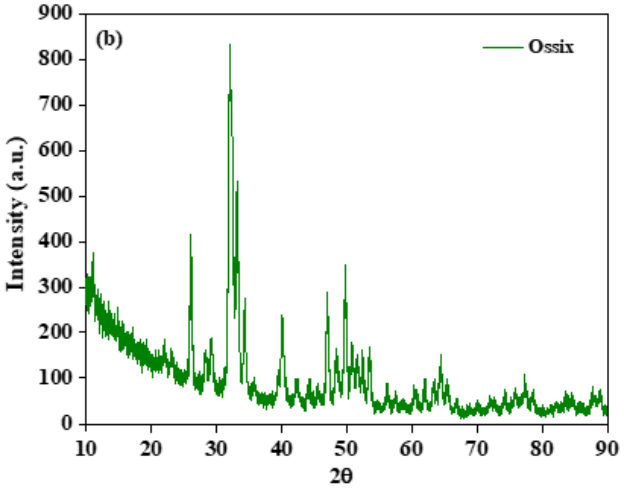
For the XRD analysis, SMCC was powdered & mounted on zero background silicon sample holder & exposed to X-rays in XRD. For SMCC, at angle 32˚ (2θ) with intensity (I) of 696 & 33˚(2θ) with I of 413; highest peak was observed and this peak was sharper as shown in graph 2. The sharp peak indicating highly crystalline material which means atoms are arranged in orderly & repeating pattern, leading to strong constructive interference of the diffracted x-rays, resulting in distinct, narrow peaks, the more crystalline the material is.



GRAPH 2 : XRD ANALYSIS OF SMCC

XRD analysis of MCS :-

For the XRD analysis, MCS was powdered & made into different samples. For that all the samples were mounted on zero background sample holder. The sharp peak of MCS shows it is more crystalline as shown in graph 3. It shows higher peak at angle 32˚ (2θ) with intensity (I) of 817 , 33˚(2θ) with I of 527 and 34˚(2θ) with I of 272 respectively. The sharp peak indicates highly crystalline material which means atoms are arranged in orderly & repeating pattern, leading to strong constructive interference of the diffracted x-rays, resulting in distinct, narrow peaks, the more crystalline the material is.



GRAPH 3 : XRD ANALYSIS OF MCS

COMPARISON

Amorphous BG are readily absorbed due to their less organized structure, making them suitable for smaller defects whereas crystalline grafts are used for larger bony defects where more stability is needed.

4. PARTICLE SIZE EVALUATION

To calculate the particle size, most intense peak in XRD pattern is identified. we have used Debye –Scherrer formula (D)

$D = K\lambda / \beta \cos\theta$

DFDBA:- Particle size of DFDBA is 2 nm. A broader peak of DFDBA indicates smaller crystal size. (Table 1)

SMCC :- Particle size of SMCC is 12 nm. A sharp peak of SMCC indicates larger crystal size. (Table 1)

MCS :- Particle size of MCS is 11 nm. A sharp peak of MCS indicates larger crystal size. (Table 1)

MATERIAL	θ	β	D(nm)
DFDBA	10.30	8.075	**1nm**
SMCC	16.12	0.69	**12.45nm**
MCS	16.09	0.74	**11.59nm**

TABLE 1 : PARTICLE SIZE

COMPARISON

The particle size of DFDBA was smaller than SMCC & MCS. Larger particles tend to provide more structural stability and may be less prone to resorption, while smaller particles can offer better space filling and potentially promote faster initial cell infiltration.

CRYSTALLINITY:

With the help of OriginLab software, area under crystalline peaks & total area under entire curve is calculated by integration method. Crystallinity % = Sum of all the areas/ total area x 100 . Crystallinity of DFDBA, SMCC, MCS was 38.3%, 74.1%, 74.5%. Higher crystallinity indicates a more organized & tightly packed mineral arrangement. While on the other hand, lower crystallinity means more porous & readily resorbable structure.

COMPARISON:

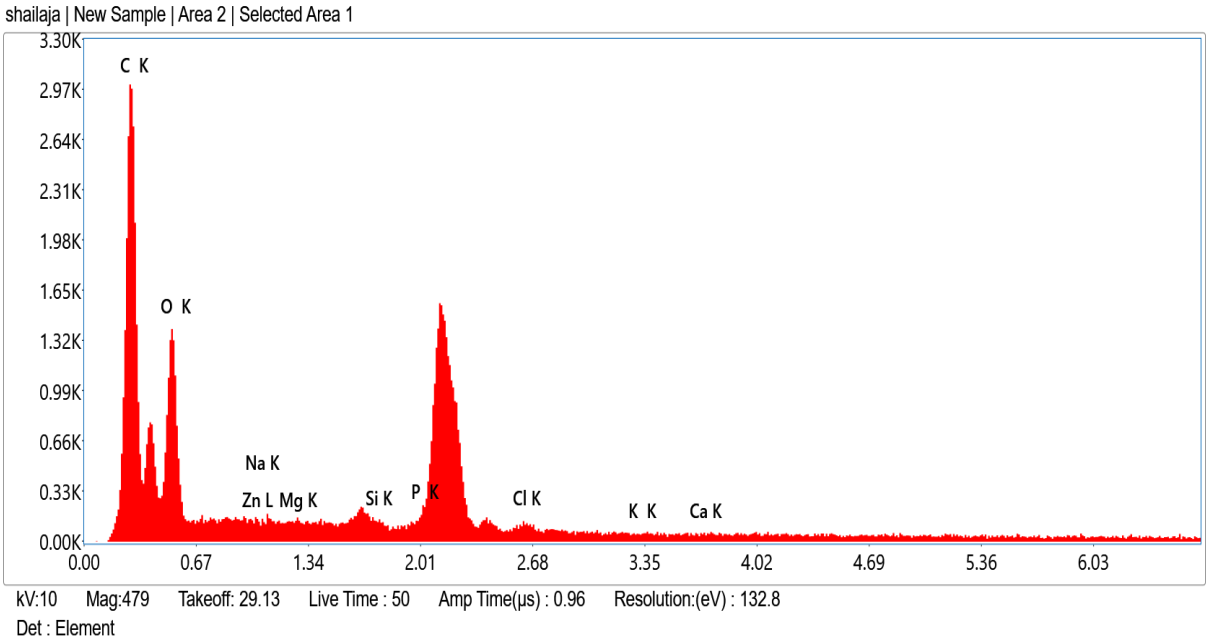
Lower crystallinity means a more porous & readily resorbable structure.

EDS MAPPING of DFDBA:

EDS mapping of the demineralized freeze-dried bone allograft substitute revealed the presence of carbon(C), and oxygen (O) and phosphorous(P) as the predominant elements, confirming its composition as a calcium phosphate-based biomaterial. The Ca and P were less distributed throughout the sample, with a Ca/P ratio of 0.02, comparable to that of hydroxyapatite. Elemental composition as seen in EDS mapping for DFDBA, is as following: (TABLE 3) (GRAPH 4).

ELEMENT	DFDBA		SMCC		MCS	
	WEIGHT%	ATOMIC%	WEIGHT%	ATOMIC%	WEIGHT%	ATOMIC%
CARBON	74.2	81.2	37.1	57.9	10.7	23.5
OXYGEN	19.8	16.3	10.6	12.5	0.0	0.0
SODIUM	0.0	0.0	0.1	0.1	0.0	0.0
MAGNESIUM	0.0	0.0	0.8	0.6	0.0	0.0
SILICON	0.3	0.1	4.0	2.7	8.2	7.7
PHOSPHOROUS	5.1	2.2	30.6	18.5	81.0	68.8
CHLORINE	0.5	0.2	-	-	-	-
POTASSIUM	0.0	0.0	-	-	-	-
CALCIUM	0.1	0.0	16.0	7.5	0.0	0.0
ZINC	0.0	0.0	0.7	0.2	0.0	0.0

TABLE 3 : EDS MAPPING



GRAPH 4 : EDS MAPPING OF DFDBA

Carbon (C): ~74.2 wt% (dominant element),

Oxygen (O): ~19.8 wt%,

Phosphorus (P): ~5 wt% (not detected)

Calcium (Ca): ~0 wt% (not detected)

Other trace elements: A very small trace of Silicon (~0.07 wt%) was detected (likely an impurity or artifact), along with **presence of Chlorine & Potassium**. No Sodium or Magnesium was detected in DFDBA.

Carbon (C), Oxygen (O), and Nitrogen (N) as the dominant elements, confirming the organic nature of the **collagenous matrix**.

Minimal Calcium (Ca) and Phosphorus (P) content, indicating a significant reduction in mineral components due to the demineralization process.

The absence of carbonate apatite, suggesting that DFDBA primarily functions as a **biological scaffold for cell attachment** rather than contributing to mechanical strength or mineral deposition.

EDS MAPPING of SMCC:

EDS analysis of Regener Oss (Regenerative bone scaffold) demonstrated a **higher Ca and P content** compared to DFDBA, confirming the presence of carbonate apatite, is as following: *(TABLE 3) (GRAPH 5)*

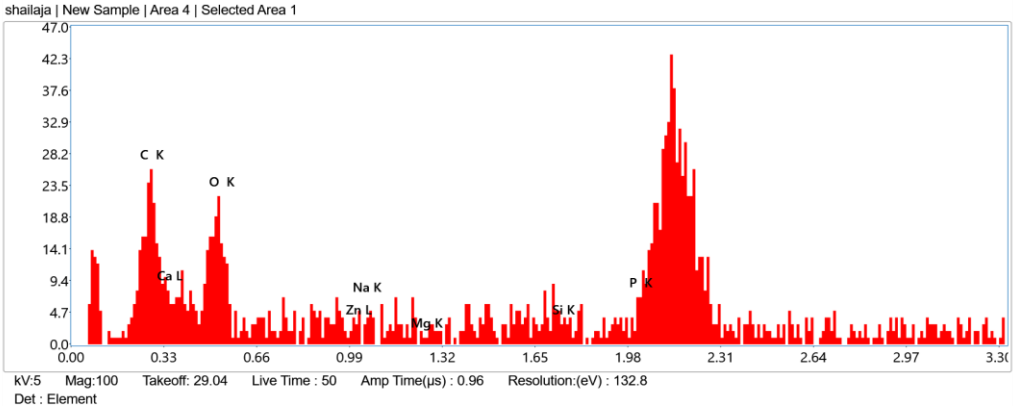
Carbon (C): ~ 37 wt% (dominant element)

Oxygen (O): ~10.6 wt%

Phosphorus (P): ~30.6 wt%

Calcium (Ca): ~16.0 wt%

Other trace elements: A very small trace of Zinc & Sodium (~0.7 wt%) was detected. **No Chlorine or Potassium** was detected in SMCC Bone graft.



GRAPH 5 : EDS MAPPING OF SMCC

EDS MAPPING of MCS:

EDS analysis of MCS (Mineralized Collagen Sponge) demonstrated a **higher P and C content** compared to DFDBA, confirming the presence of calcium phosphate based biomaterial. EDS analysis highlights the composite nature of the scaffold, is as following: (TABLE 3) (GRAPH 6)

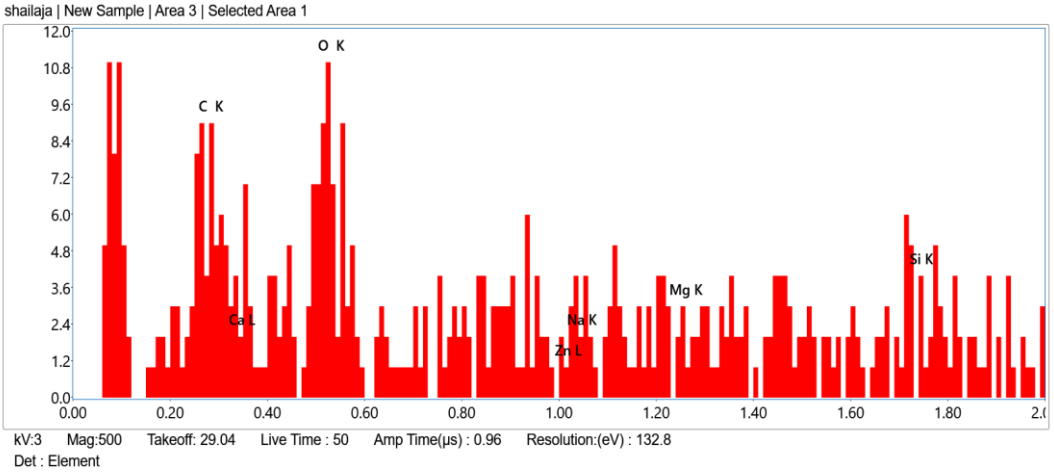
Carbon (C): ~ 10.7 wt% (dominant element)

Oxygen (O): ~0 wt%

Phosphorus (P): ~81 wt%

Calcium (Ca): ~0 wt%

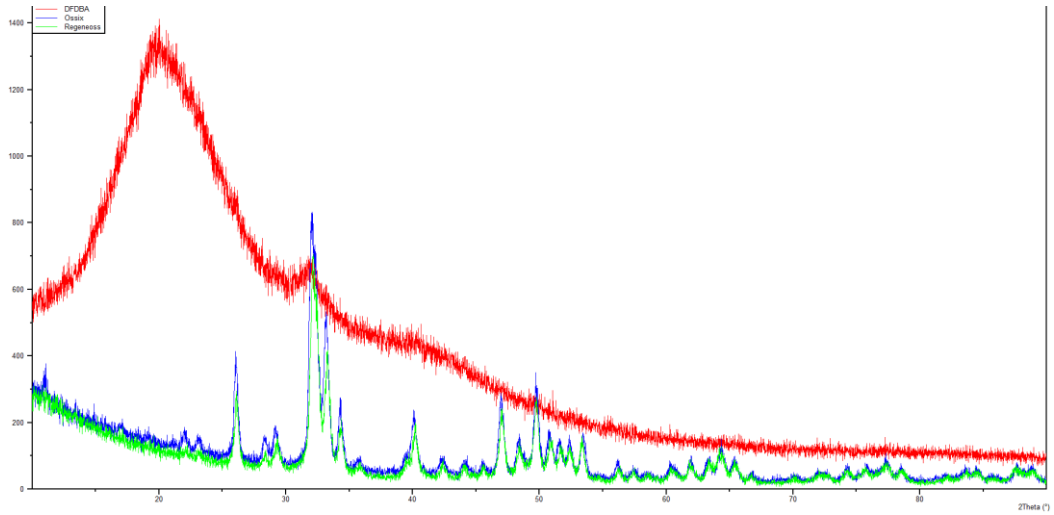
Other trace elements: A very small trace of Silica & Sodium which are known to enhance bioactivity. **No Chlorine or Potassium** was detected in MCS Bone graft.



GRAPH 6 : EDS MAPPING OF MCS

COMPARISON OF DFDBA BONE ALLOGRAFT & SMCC BONE GRAFT FROM SEM AND EDS ANALYSIS: (GRAPH:7)

1. DFDBA: Primarily composed of an organic collagen network, making it suitable for osteogenic cell infiltration but lacking inherent mineral stability.
2. SMCC (RegenerOss™ Synthetic): A biomimetic mineralized scaffold, integrating carbonate apatite crystals within a Type-I bovine collagen matrix, enhancing bioactivity, osteoconductivity, and mechanical stability.
3. The SMCC may offer superior mechanical reinforcement and bone regeneration potential compared to DFDBA, making it a promising alternative for clinical applications requiring enhanced osteointegration.
4. The MCS shows enhanced osteointegration as well as bioactivity.



GRAPH 7 : COMPARATIVE EVALUATION OF ALL THE 3 SAMPLES

5. DISCUSSION

Periodontitis has been identified as a multifactorial disease that encompasses reversible loss of the periodontium and interactions among microbes, ultimately resulting in attachment loss. The objectives of non-surgical (Phase I) treatment focus on halting disease progression and eliminating associated signs and symptoms. Conversely, in phase II, this material aims to restore the periodontium to its normal height.[8] Recent advancements in materials have emerged, incorporating hydroxyapatite crystals & collagen. Several studies conducted in the past have demonstrated its association with systemic diseases, including those affecting the cardiovascular system, diabetes mellitus, pulmonary conditions, and complications related to pregnancy.[9,10] Therefore, researchers need to find ways to reduce the damage caused by this infection by managing it and using basic medical principles that ensure proper space, keep wounds stable, and promote healing. [11,12] Research indicates that the application of guided tissue regeneration (GTR) in conjunction with DFDBA results in superior regenerative outcomes compared to the use of autogenous tissue alone. [13,14] While others held differing opinions based on its osteoinductive and osteoconductive properties.[15-17] They had also showed that they facilitates mesenchymal cell migration, attachment, and osteogenesis within well-vascularized bone tissue. [18] While other, studies indicate that commercially available DFDBA can induce new bone formation in in vivo studies. However, there is considerable variability in clinical responses, attributed to differences in preparation methods at commercial bone banks and variations in host responses.[14, 19] Although there were differences in processing methods according to other studies, they do not provide an accurate conclusion regarding to the variability in bone induction around the defect.[20] Currently, DFDBA from the American Association of Tissue Banks is regarded as generally safe for regenerative procedures, particularly when considering the age of the donor. [18] A study indicates that when DFDBA is utilized in a particulate form, the size of the particles is a significant variable influencing the success of the procedure. [18]

In a study, XRD was utilized for a variety of purposes, including the identification of unknown crystalline substances, such as minerals and inorganic chemicals; the identification of fine-grained minerals, such as clays and mixed-layer clays; the determination of unit cell lengths; the evaluation of sample purity; the determination of modal amounts in quantitative form; the characterization of thin film samples; and the measurement of textural characteristics. [21] In addition, a study discovered that the various CBRs were composed of calcium phosphate-based minerals that had a carbonate apatite type of structure. This structure is virtually identical to that of actual bone, which is composed of type I collagen. [22]

6. LIMITATION OF THE STUDY

1. DFDBA shows better arrangement of the pores (honeycomb appearance) which is beneficial for predictable bone growth, controlled degradation & mechanical stability. Whereas collagen based regenerative material, is better in terms of cell attachment , bioactivity & capillary infiltration.
2. The surface roughness is higher in SMCC & MCS, thereby resulting in enhanced osteoblast adhesion, proliferation, osteoconductivity, osseointegration, mechanical interlocking, stability, vascularization and finally, nutrient diffusion.
3. Interconnectivity & pore shapes were found better arranged but collagen sponge was less arranged in DFDBA, while on the other hand, cell migration, nutrient transport, interconnected pores was better for SMCC & MCS.
4. DFDBA has round/spherical uniform pores which helps in migration, fluid diffusion, vascular infiltration but reduce

mechanical interlocking. While, SMCC& MCS had interconnected pores, mechanical interlocking, cell attachment, oxygen diffusion and crucial for larger grafts.

7. CONCLUSION

DFDBA exhibited reduced roughness while demonstrating increased porosity, which may facilitate osteoconduction. On the other hand, SMCC and MCS had a rougher surface and a more porous structure, which could help cells stick better, allow nutrients to move more easily, and improve bone growth. The differences in mineral makeup, especially the silicon found in SMCC and MCS and the oxygen in DFDBA, show the unique beneficial qualities of these minerals. Thus, it is essential to select the background material in accordance with specific clinical requirements. SMCC demonstrates enhanced bioactivity and integration attributed to its calcium-to-phosphorus ratio and open porosity. DFDBA remains a viable option for scenarios that require enhanced porosity and osteoconductivity. More studies are needed to test these materials in living organisms to confirm their long-term effectiveness and to tailor their use in gum and implant dentistry.

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