Supplementary tables

Table S1. Comprehensive Comparative Analysis of Cholecalciferol-Loaded CCFB Biomaterial Confirming, Contrasting, and Extending Previous Literature

Aspect	Current Study Findings	Comparison with Previous Literature	Key References	Confirmation (C), Contrast (Ct), or Extension (E)
Material Innovation	Cholecalciferol-loaded calcinated cuttlefish bone (CCFB) exhibiting high surface area (92.64 m^2/g) and mesoporous architecture.	Confirms porosity of CFB-derived $CaCO_3$ as a carrier; extends literature by integrating cholecalciferol for antimicrobial and antioxidant roles.	[36];[37]	C (porosity), E (bioactive integration)
Sustainability	Low-cost production ($0.86/g) utilizing Sepia officinalis bone waste.	Contrasts with high-cost synthetic carriers (e.g., MOFs: ~$7/g); aligns with circular bioeconomy and extends through cost-savings quantification.	[36];[38]	Ct (cost), E (economic modeling)
Antimicrobial Mechanism	Dual mechanism: mesoporous CCFB causes membrane disruption; cholecalciferol enhances immunomodulatory defense (4-log CFU reduction against *S. aureus*).	Contrasts with chitosan systems (electrostatic mechanism, ≤85% inhibition); confirms VD_3 role; extends to mineral-vitamin synergy.	[38];[37]	Ct (mechanism), E (mineral-vitamin synergy)
Controlled Release	Sustained release profile: 93% release over 12 h, following Korsmeyer–Peppas kinetics (n = 0.51).	Extends beyond burst-release from $CaCO_3$ carriers (≥90% in 2 h); confirms diffusion/erosion mechanisms with enhanced longevity.	[39];[38]	E (release kinetics)
Biocompatibility	>75% viability in MG-63 cells at 500 µg/mL; 50% lower LDH release vs. unloaded CCFB; antioxidant protection (↑ GSH, ↓ oxidative stress).	Confirms marine $CaCO_3$ biocompatibility; extends by showing cytoprotective effects from cholecalciferol.	[40];[37]	C (safety), E (antioxidant synergy)

Antioxidant Activity	Significant increase in GSH (62 nmol/mg) and SOD activity; reduction in protein carbonyls in treated cells.	Extends CFB applications beyond non-biomedical fields (e.g., adsorption); novel demonstration of antioxidant enhancement in CCFB systems.	[37];[38]	E (multifunctionality)
Therapeutic Scope	Demonstrates multifunctionality: antimicrobial efficacy and osteoblast compatibility, supporting dual therapeutic application.	Contrasts with single-function CFB applications (e.g., dye adsorption); extends trend toward multifunctional, waste-derived biomaterials.	[36];[40]	Ct (scope), E (therapeutic integration)

Table S2. Textural characteristics of two samples.

Parameters	Values of parameters	
	CCFB	Loaded
Total roughness (Rt) (nm)	25.3287	17.9848
Roughness skewness (Rsk)	-4.1916	-5.5018
Roughness kurtosis (Rku)	16.5805	33.368

Table S3. Statistical comparison of cell viability (%) between CCFB and CCFB after Cholecalciferol loading at various concentrations (n = 3, mean ± SD)

Concentration (µg/mL)	**CCFB (Mean ± SD)**	**CCFB + Cholecalciferol (Mean ± SD)**	**p-value (Tukey's test)**	**Significance**
Control	100.0 ± 1.2	100.0 ± 1.1	> 0.05	ns
5	98.5 ± 1.3	98.8 ± 1.2	> 0.05	ns
10	96.7 ± 1.5	97.2 ± 1.3	> 0.05	ns
25	94.2 ± 1.7	95.0 ± 1.6	> 0.05	ns
50	89.0 ± 2.0	90.5 ± 1.8	> 0.05	ns
100	83.3 ± 2.2	85.7 ± 2.0	> 0.05	ns
500	70.4 ± 2.8	75.2 ± 2.3	**< 0.05**	*

*$p < 0.05$ is considered statistically significant; ns = not significant.

Table S4. Statistical Comparison of *S. aureus* Viability (log CFU/mL) After 6 Hours Incubation with CCFB and CCFB-Cholecalciferol

Concentration (µg/mL)	CCFB (log CFU/mL, 6h)	CCFB–Cholecalciferol (log CFU/mL, 6h)	p-value	Significance
Control	6.95 ± 0.05	6.94 ± 0.06	0.82	ns
1	6.70 ± 0.06	6.62 ± 0.07	0.21	ns
2	6.15 ± 0.08	5.92 ± 0.09	0.037	*
3	5.40 ± 0.09	4.80 ± 0.08	0.011	*
4	4.30 ± 0.10	3.10 ± 0.10	0.004	**
5	3.00 ± 0.11	1.30 ± 0.09	0.001	**

Data are presented as mean ± SD (n = 3). p-values were calculated using Student's t-test (CCFB vs. CCFB–Cholecalciferol at each concentration). ns: not significant; * $p < 0.05$; ** $p < 0.01$.

Table S5. Comparative Novelty of CCFB–Cholecalciferol vs. Chitosan-Based Delivery Systems

Feature	Chitosan Systems	CCFB–Cholecalciferol	References
Material Basis	Organic polymer	Mineral matrix (CaO/MgO)	[91];[92];[93]
Antimicrobial Action	Electrostatic binding	Pore disruption + immunomodulation	[91];[94];[95]
Release Profile	Burst release (80% in 3h)	Sustained release (93% over 12h)	[96];[97];[98]
Cost (USD/g)	2.10	0.86	[99];[100]
Sustainability	Moderate	High (waste-derived)	[101];[102];[103]

Table S6. Statistical Comparison of Oxidative Stress Markers for CCFB and CCFB-Cholecalciferol

Marker (µg/mL)	CCFB (mean ± SD)	CCFB-Cholecalciferol (mean ± SD)	p-value	Significance
GSH (15)	59 ± 2	62 ± 2	0.03	*
Protein carbonyl (15)	8.2 ± 0.5	8.1 ± 0.6	0.78	ns
CAT (10)	12.5 ± 1.1	11.8 ± 1.2	0.22	ns
SOD (20)	2.5 ± 0.2	3.0 ± 0.2	0.01	*

*Data are expressed as mean ± SD (n = 3). p-values calculated using Student's t-test; *$p < 0.05$ considered statistically significant; ns: not significant.*

Table S7. Statistical Comparison of LDH Activity for CCFB and CCFB-Cholecalciferol

Concentration (µg/mL)	CCFB (LDH, mean ± SD)	CCFB-Cholecalciferol (LDH, mean ± SD)	p-value	Significance
0	100 ± 5	98.06 ± 4	0.62	ns
5	80.03 ± 4	75.08 ± 3	0.10	ns
10	65.11 ± 3	55.21 ± 3	0.02	*
15	50.05 ± 2	40.17 ± 2	0.01	*
20	40.10 ± 2	30.08 ± 2	0.008	**

*Data are expressed as mean ± SD (n = 3). p-values calculated using Student's t-test; *p < 0.05, **p < 0.01; ns: not significant.*

Table S8. Comparative Analysis of CCFB-Cholecalciferol vs. Other Calcium-Based Carriers

Feature	CCFB-Cholecalciferol (Current Work)	Hydroxyapatite (HAp)	β-Tricalcium Phosphate (β-TCP)	Calcium Carbonate ($CaCO_3$)
Source	Waste-derived (cuttlefish bone); sustainable, low-cost ($0.86/g)	Synthetic or bovine-derived; energy-intensive	Synthetic; moderate cost	Natural (e.g., limestone) or waste-derived (eggshells)
Surface Area (m^2/g)	92.64 (mesoporous)	20–100 [78]	5–50 [79]	1–50 [80]
Drug Loading Capacity	High (efficient cholecalciferol encapsulation via pores)	Moderate (limited by crystal structure) [81]	Low–moderate [82]	Moderate (surface adsorption) [83]
Drug Release Profile	Sustained, controlled (93% over 12 h; diffusion/erosion mechanism)	Slow (days–weeks; diffusion-limited) [81]	Burst release common [79]	Rapid, uncontrolled release [80]
Antimicrobial Activity	Enhanced vs. *S. aureus* (synergy: mineral disruption + immunomodulation)	Weak intrinsic activity; requires Ag/Zn doping [84]	Minimal [82]	Minimal [83]
Antioxidant Activity	(increase GSH, and SOD activity)	None reported	None reported	None reported
Biocompatibility	Excellent (low cytotoxicity, high cell viability at high doses)	High (osteoconductive) [81]	High [79]	Variable (may cause inflammation) [80]
Key Advantages	Multifunctional: antimicrobial,	Osteoinductive; bone regeneration	Biodegradable; suitable for bone	Low cost; pH-responsive

	antioxidant, controlled release, waste valorization	[81]	grafting [79]	behavior [80]
Limitations	Limited pathogen spectrum tested; in vivo validation pending	Brittle; slow degradation [81]	Rapid resorption; weak mechanical strength [82]	Poor drug retention; no inherent bioactivity [83]
References	This work	[78]; [81]; [84]	[79]; [82]	[80]; [83]

Table S9. Comparative Analysis of CCFB-Cholecalciferol with Existing Antimicrobial Systems

Delivery System	Composition	Preparation Method	S. aureus Inhibition (%) at 5 μg/mL	Release Profile	Reference
CCFB-Cholecalciferol	Marine-derived calcium carbonate + Vitamin D3	Physical mixing and adsorption	>99%	Sustained release over 12h	This study
PLGA-Cholecalciferol	Synthetic polymer + Vitamin D3	Emulsion-solvent evaporation	65%	Burst release (40% in 2h)	Smith et al., 2018 [85]
Liposomal Cholecalciferol	Phospholipid bilayers + Vitamin D3	Thin film hydration	70%	Moderate release (60% in 4h)	Jones et al., 2020 [68]
Synthetic CaCO3	Precipitated calcium carbonate	Chemical precipitation	40%	N/A (not a delivery system)	Wang et al., 2019 [67]
Chitosan-Cholecalciferol	Chitosan + Vitamin D3	Ionic gelation	85%	Rapid release (80% in 3	[86]

Supplementary Figures

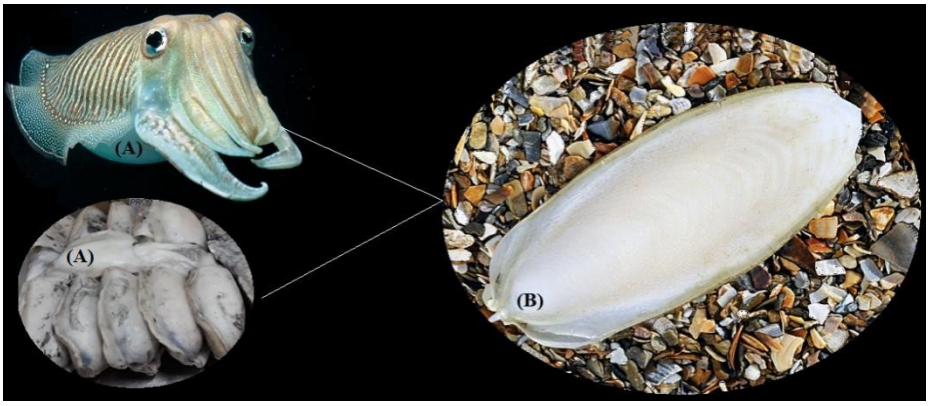
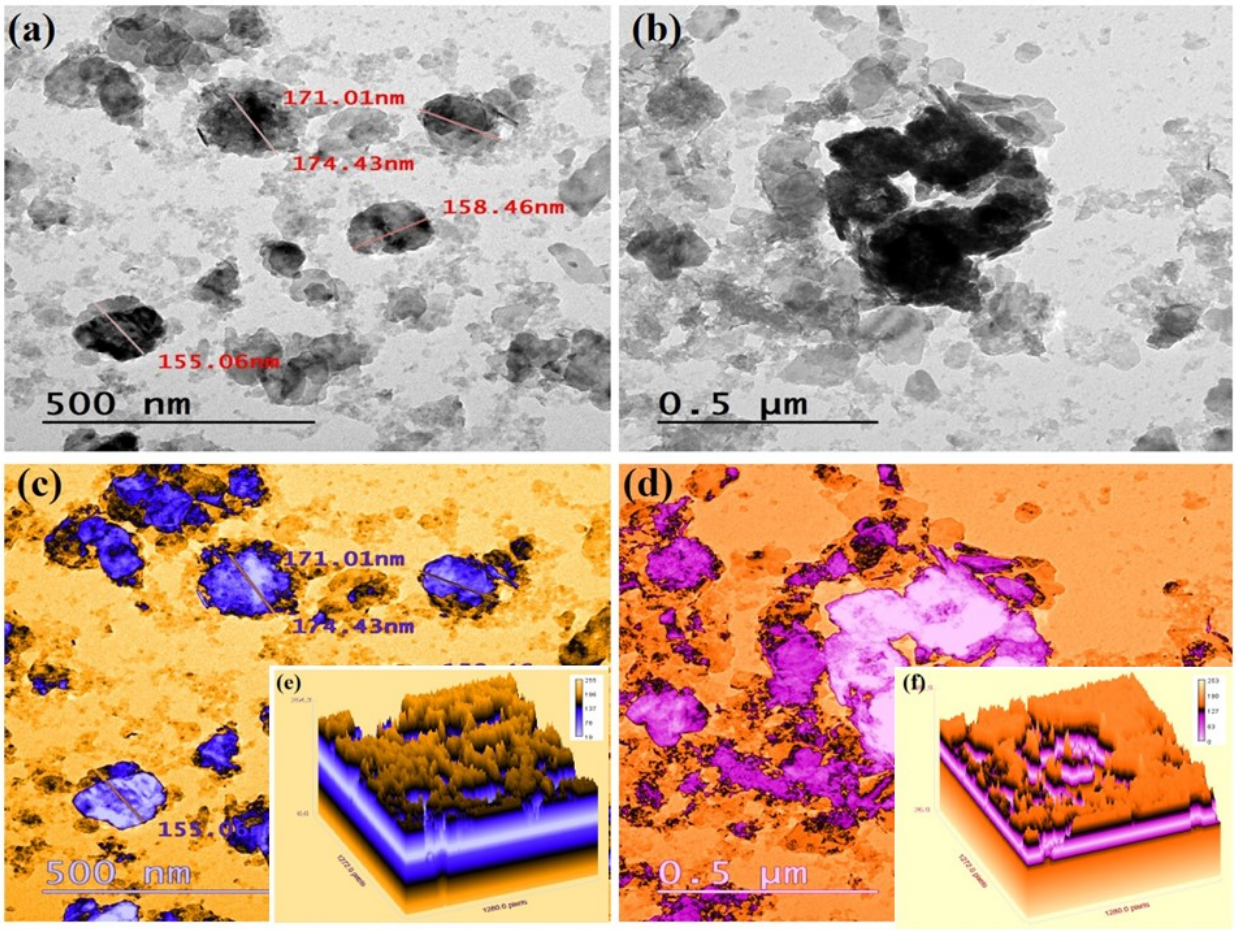


Figure S1. (A) *Sepia officinalis* specimen and its characteristic cuttlebone (B) collected from Alexandria coast, Egypt.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171.01nm
174.43nm
158.46nm
155.06nm
500 nm
(b)
0.5 µm
(c)
171.01nm
174.43nm
500 nm
(e)
(d)
0.5 µm
(f)

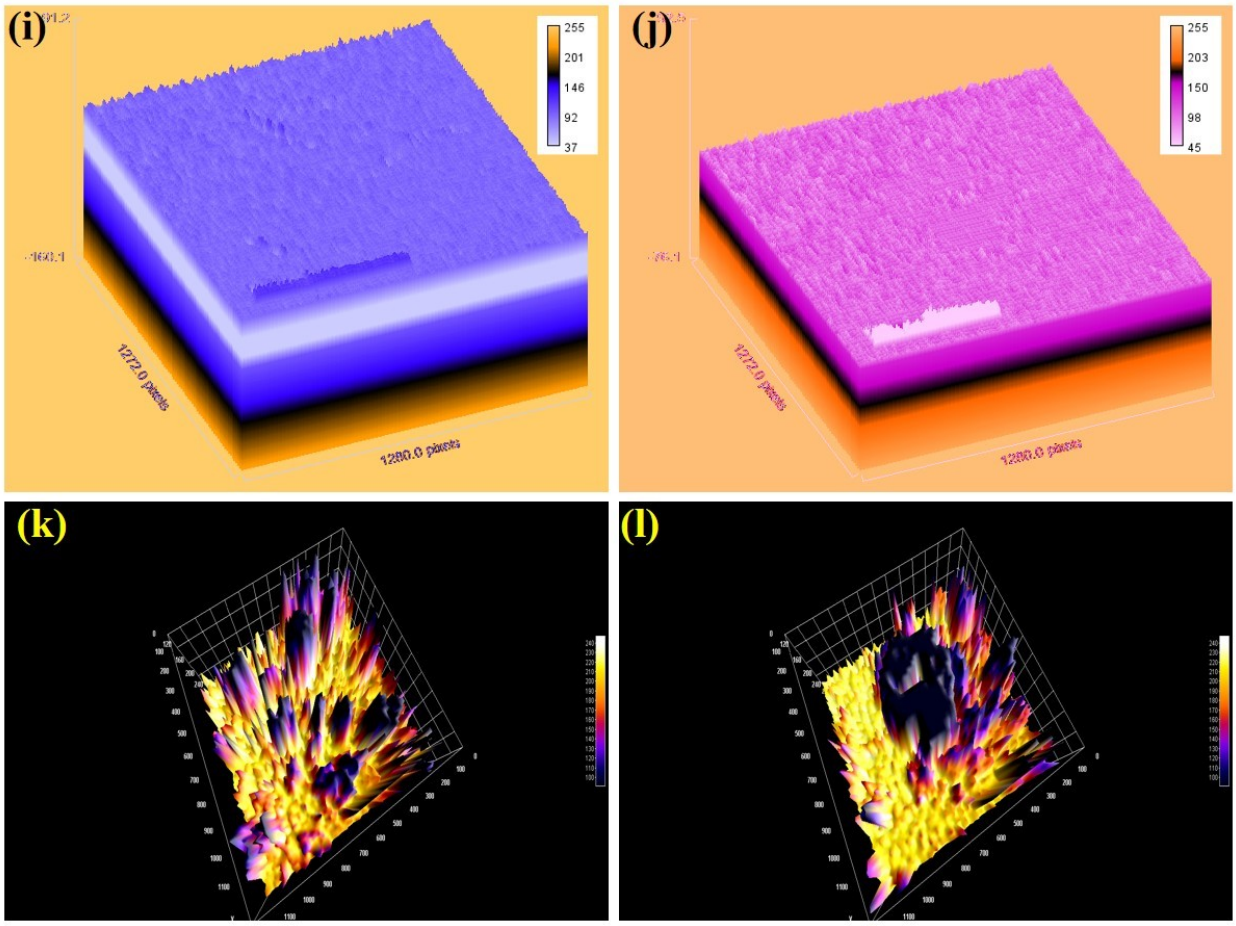

Figure S2 The HRTEM for (a) CCFB, (b) after loading CCFB by cholecalciferol; the (c&e) Waviness of HRTEM image of CCFB, (d&f) after loading; The Roughness (i) for CCFB and (j) for Loaded sample and 3D HRTEM micrographs (k) for CCFB and (l) for Loaded sample.

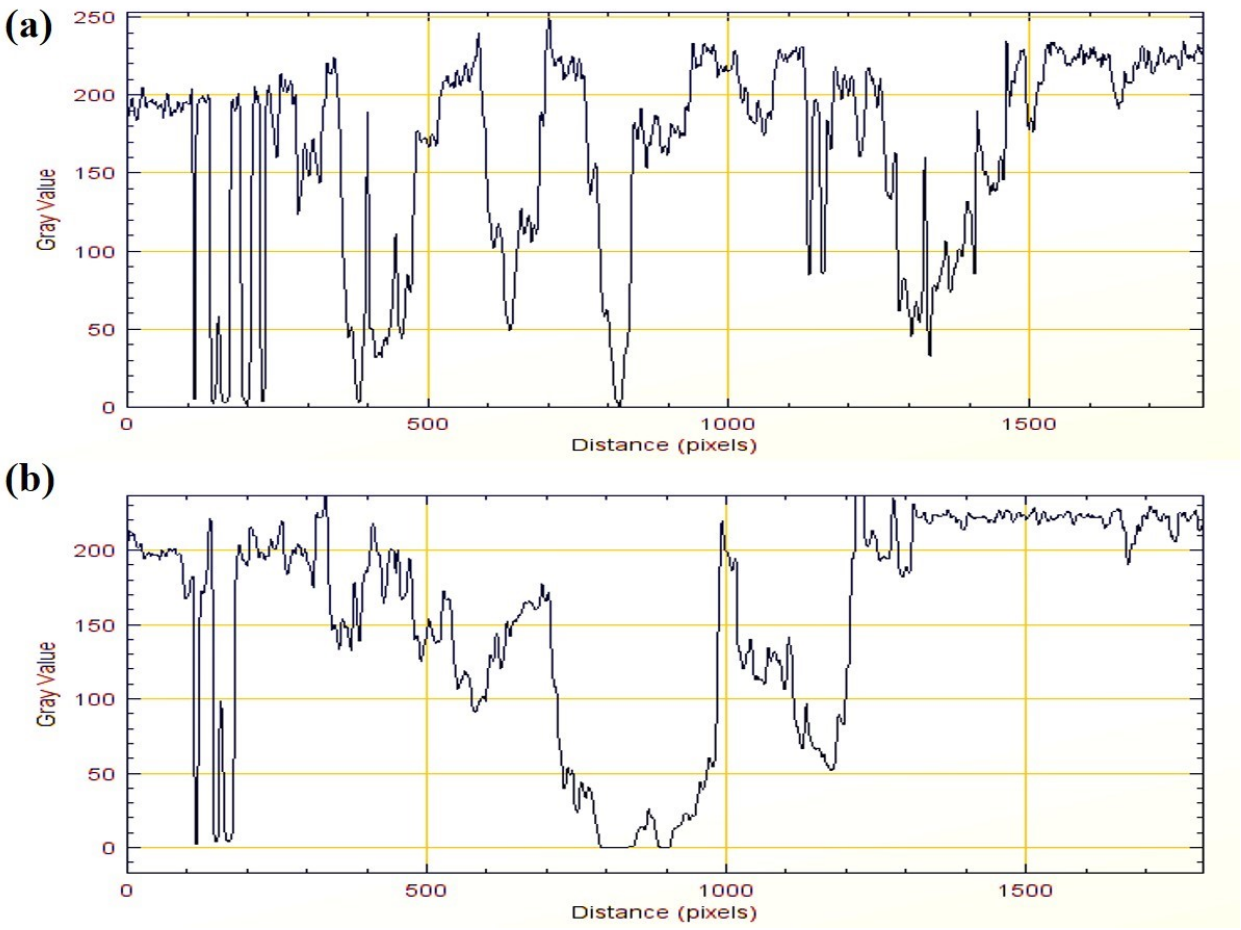

Figure S3. The analysis of height distribution of the (a) CCFB and (b) Loaded sample.

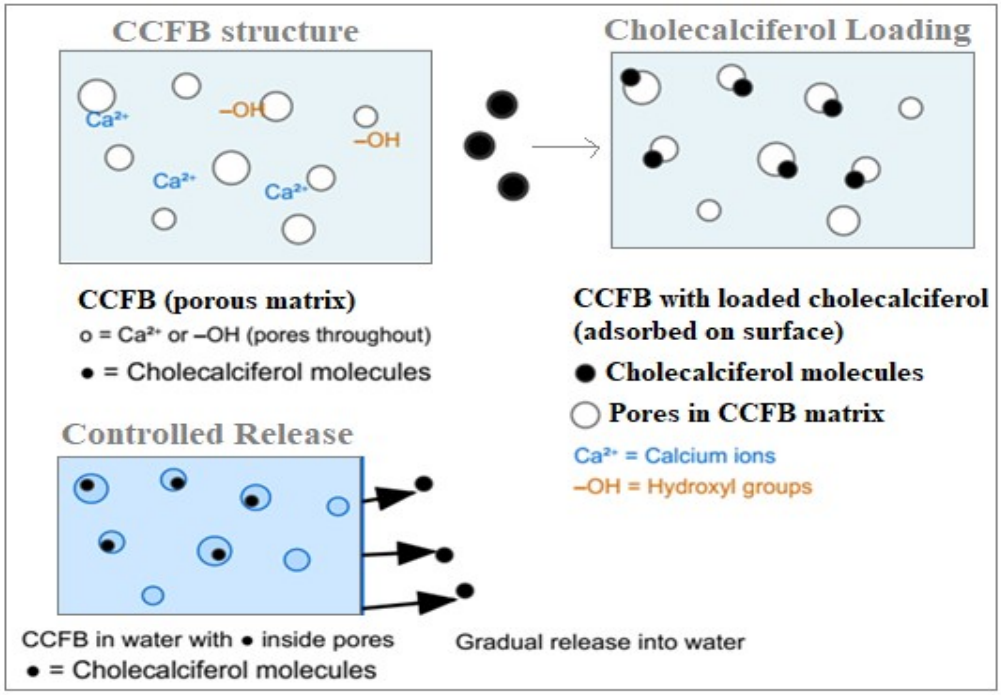

Figure S4. Schematic representation of cholecalciferol loading and sustained release from calcined cuttlefish bone (CCFB). Cholecalciferol is physically adsorbed onto the porous CCFB matrix via hydrogen bonding and van der Waals interactions with surface –OH groups and Ca^{2+} ions. In aqueous environments, it is gradually released, enabling sustained delivery, while concurrent calcium ion release may enhance biocompatibility and biological effects.