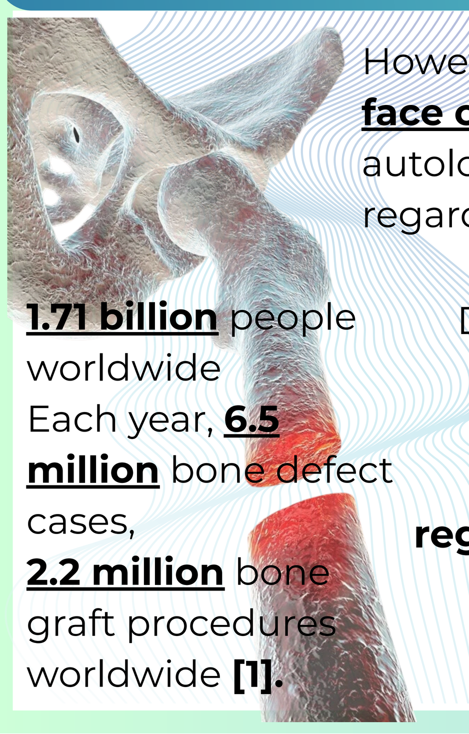
Mineralised Biopolymer-Based Scaffolds Containing Natural Bioactive Compounds for Bone Tissue Engineering

Thanh-Nghi Do Ly, Khanh Quang Pham, Giang V.H. Phan
Biomaterials and Nanotechnology Research Group, Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam

ABSTRACT

In this study, an artificial extracellular matrix scaffold was developed for **non-healing bone defects** using **natural polymers** (gellan gum, carboxymethyl chitosan, and bacterial cellulose). **In situ mineralization** deposited mineral crystals on the framework, enhancing structural and mechanical properties while mimicking bone's organic–inorganic phase. **Bioactive compounds** from *Cissus quadrangularis* were incorporated during mineralization. Characterizations of pore structure, hydroxyapatite formation, and controlled release of organic molecules highlight the scaffold's **potential for bone tissue engineering** and regenerative medicine.

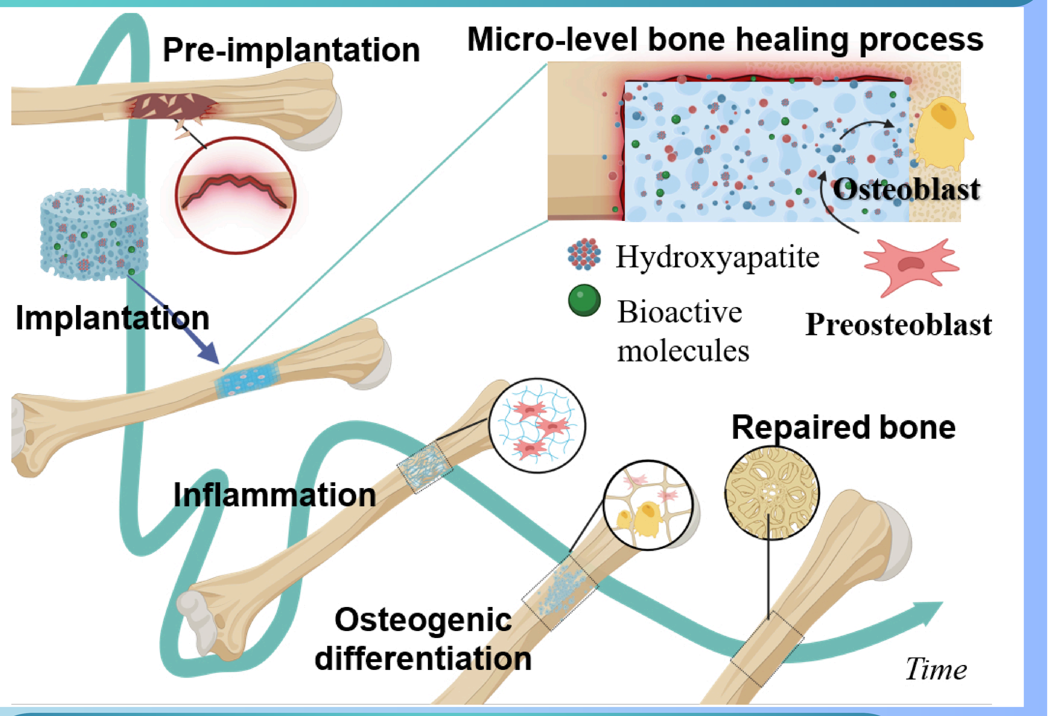
INTRODUCTION



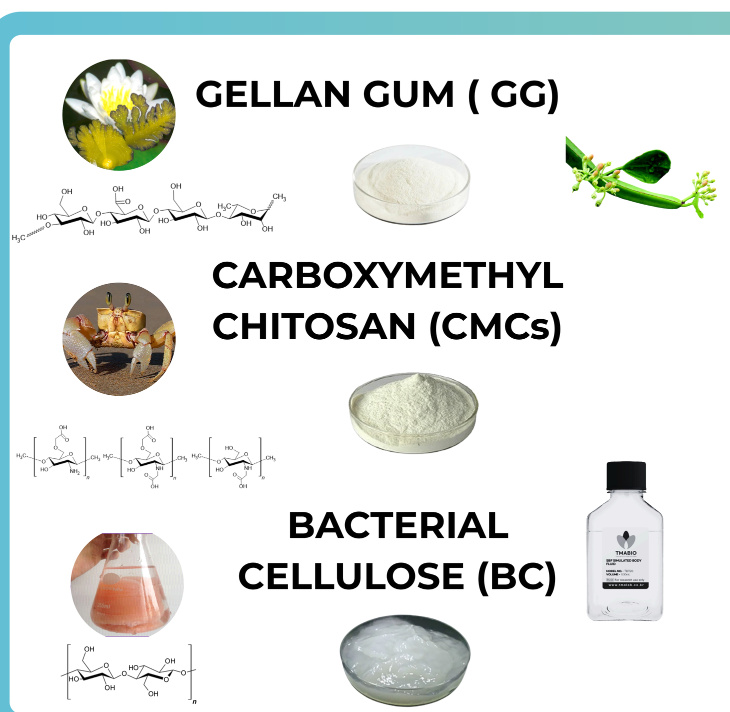
<u>**1.71 billion**</u> people worldwide
Each year, <u>**6.5 million**</u> bone defect cases,
<u>**2.2 million**</u> bone graft procedures worldwide **[1]**.

However, <u>**the current methods all face certain limitations**</u>, although autologous bone grafting is still regarded as the gold standard **[2]**.

Due to these challenges, **current research has shifted toward advanced and safe materials capable of promoting bone regeneration**. This pressing clinical demand has accelerated the development of bone tissue engineering

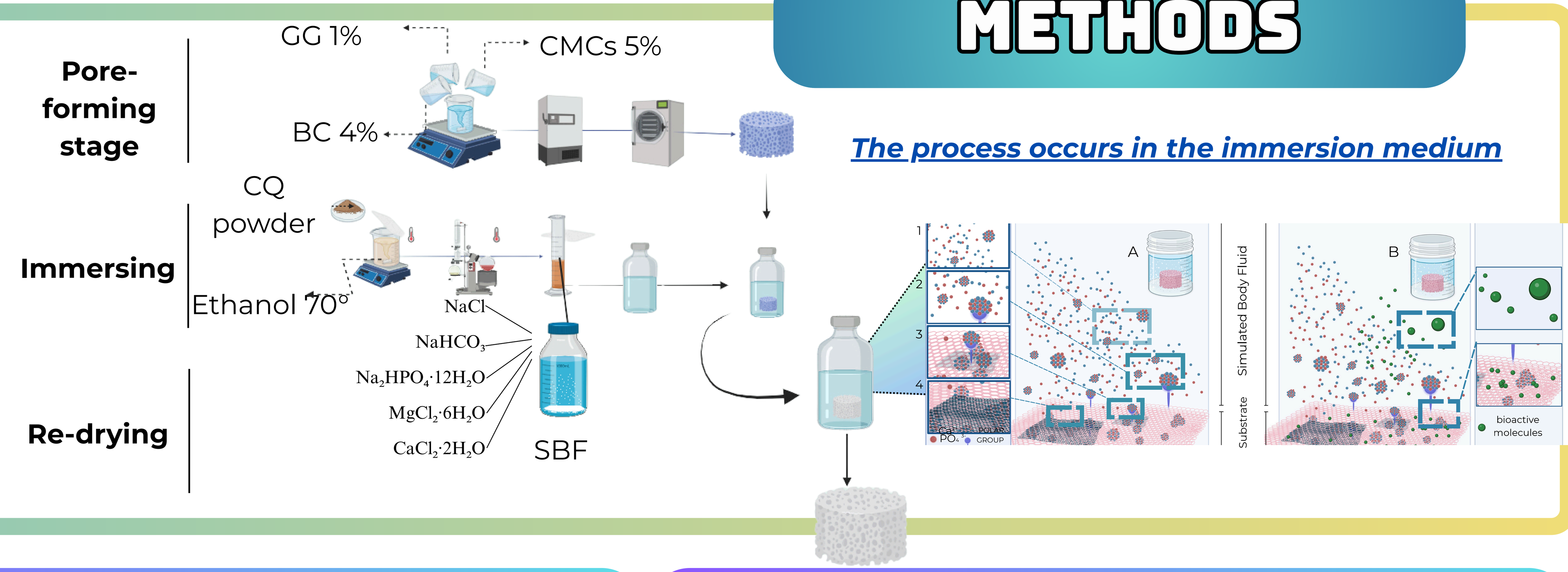


METHODS



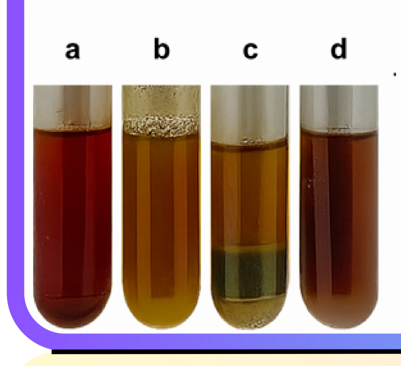
Extract of *Cissus quadrangularis* (CQ) It has long been used to promote bone and tissue healing and provide various therapeutic benefits. **[3]**.

Simulated body fluid (SBF), Its plasma-like ionic composition induces carbonate apatite formation on material surfaces **[4]**.



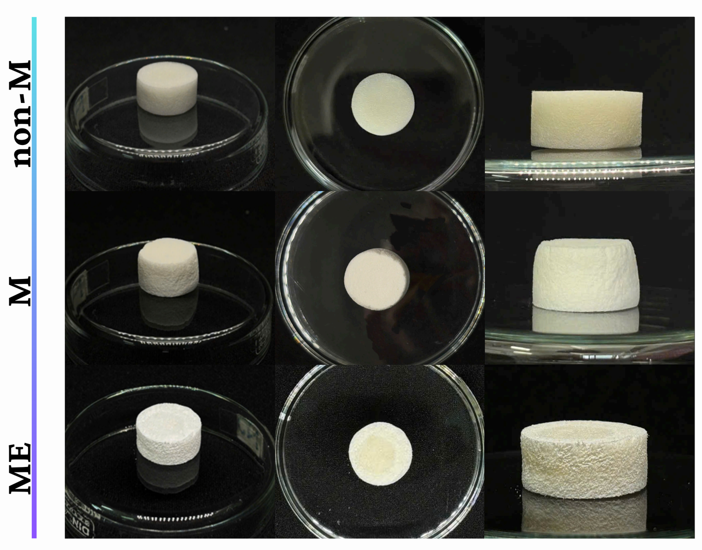
RESULTS

I. Phytochemical screening of Cissus quadrangularis extract



Phytoconstituents	Inference
Alkaloids	++
Flavonoids	+
Phenolic compounds	++
Steroids	-

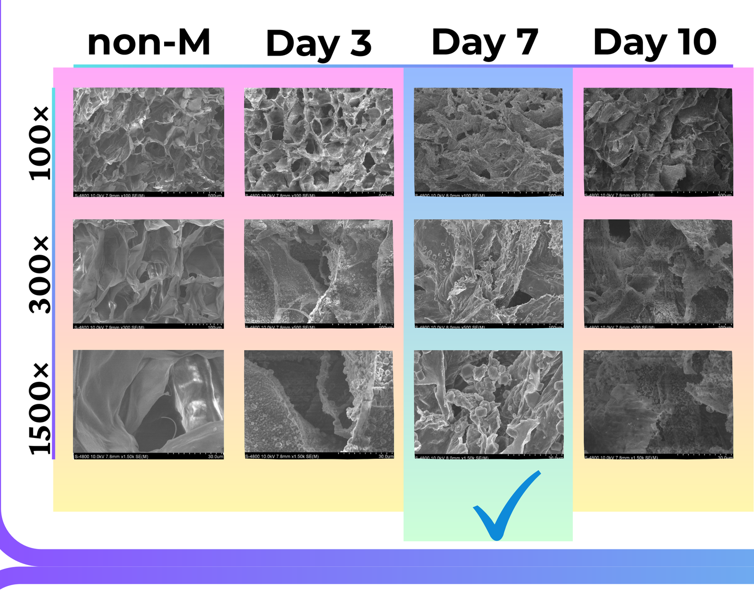
II. Macroscopic appearance of scaffolds after mineralization

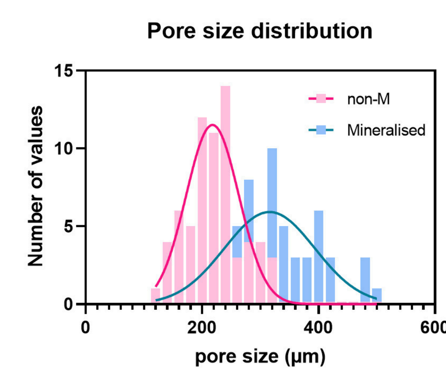


Increased surface roughness after mineralization

non-M: Non-mineralized
M: Mineralized
ME: CS-enriched mineralized

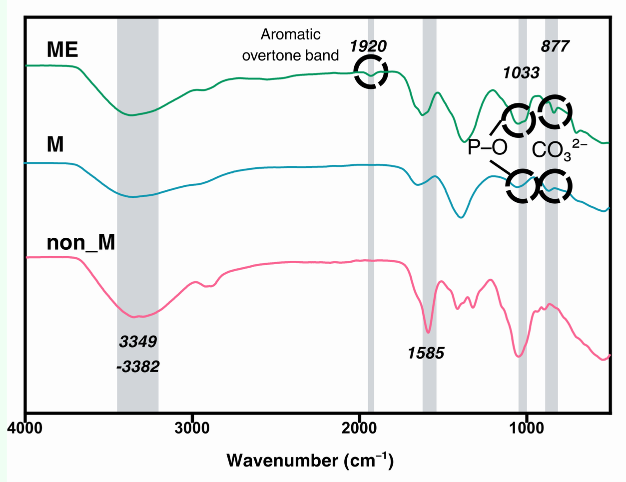
III. Time-dependent monitoring of the mineralization process

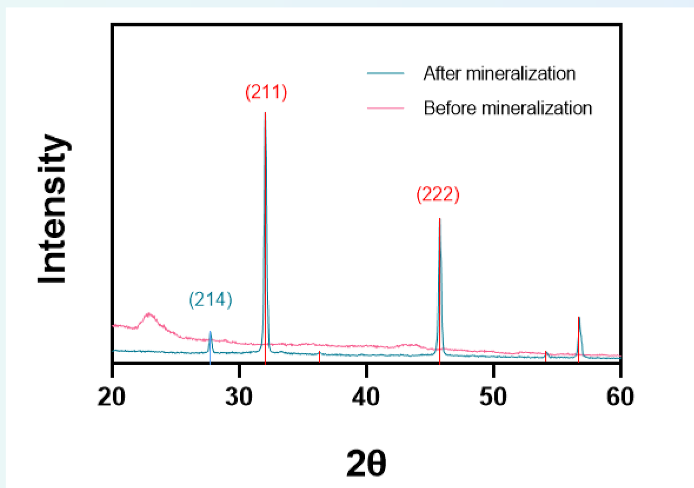


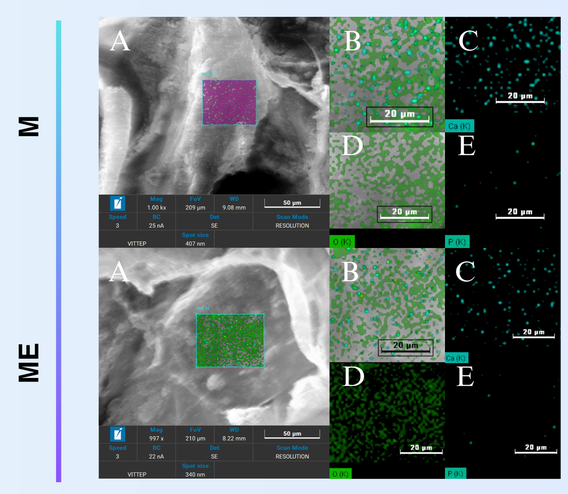


At day 7, the mineral crystals were well-developed

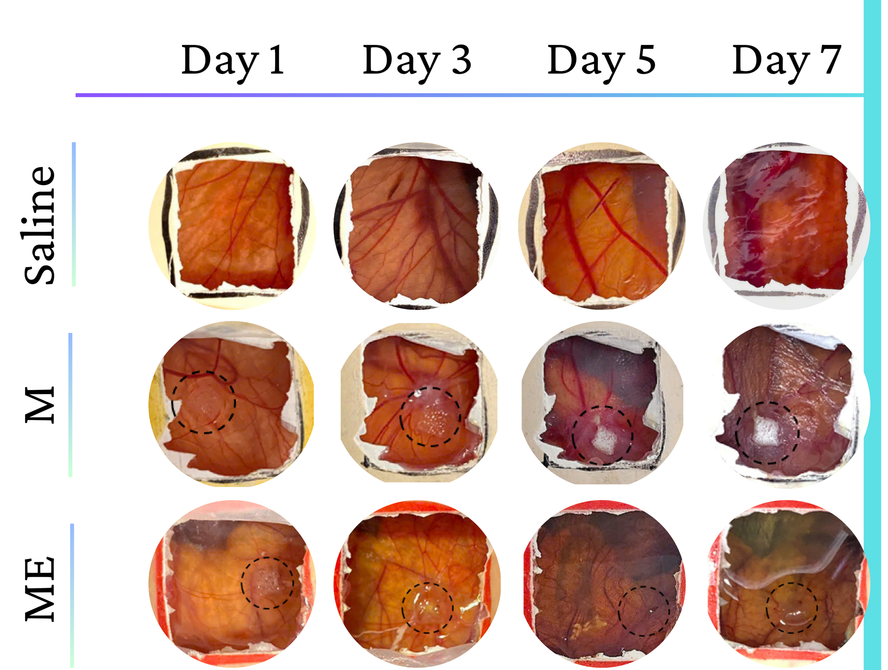
IV. STRUCTURAL AND CHEMICAL CHARACTERIZATION



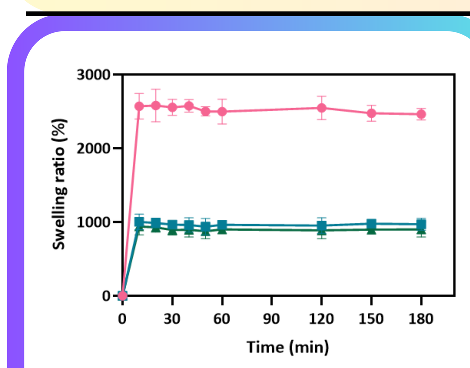


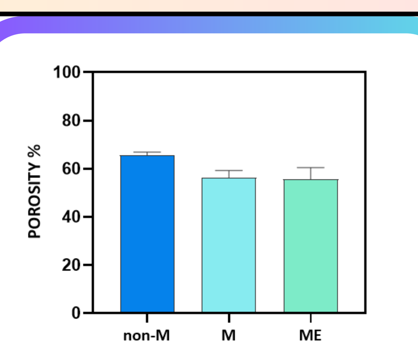


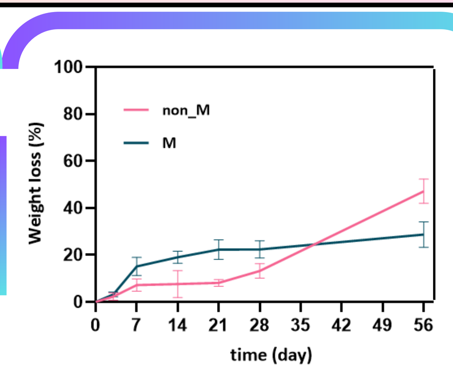
The results of **FTIR**, **XRD**, and **EDS mapping** analyses **indicate** the formation of the **hydroxyapatite** phase, along with the <u>presence</u> of **organic molecules** uniformly **distributed** on the sample surface.



The chick CAM assay results demonstrated that both M and ME samples **maintained good biocompatibility throughout the 7-day** observation period.

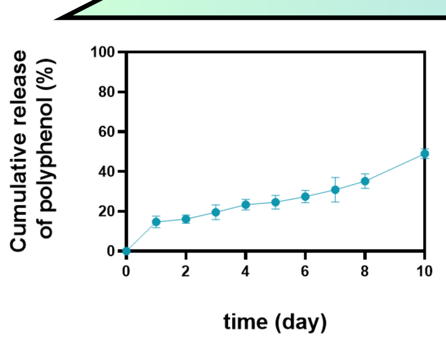




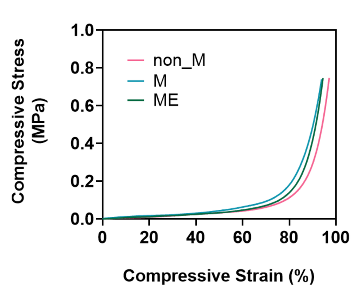


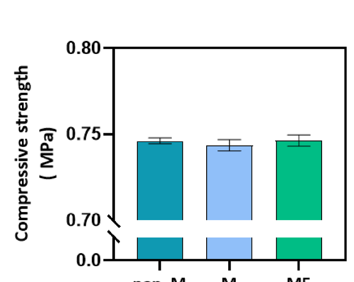
The results of **swelling**, **porosity**, and **biodegradation** analyses demonstrate that the adjustment of the inorganic phase influences the material properties, which collectively ensure the suitability of the scaffold for biomedical applications.

V. PHYSICOCHEMICAL & MECHANICAL PROPERTIES



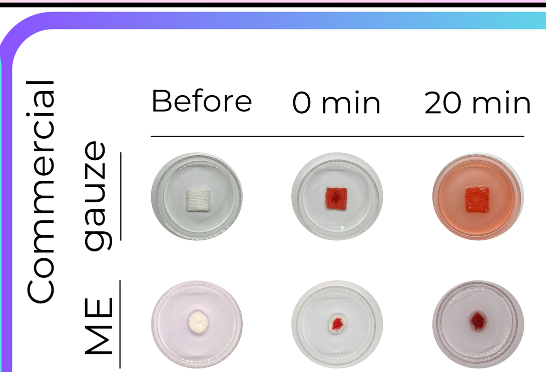
The release of bioactive molecules from the CQ extract



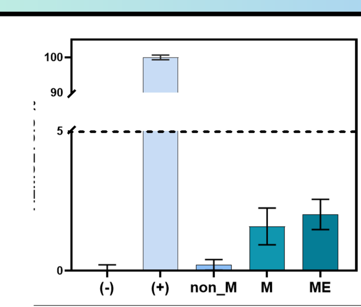


Stress–strain analysis revealed increased brittleness after mineralization, while **compressive strength** remained largely unchanged, indicating that strength was mainly dictated by the porous structure.

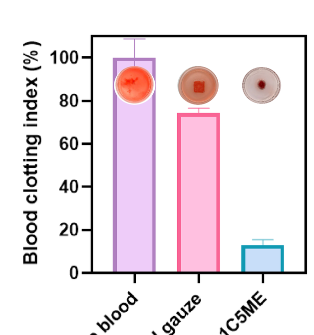
VI. BIOLOGICAL EVALUATION

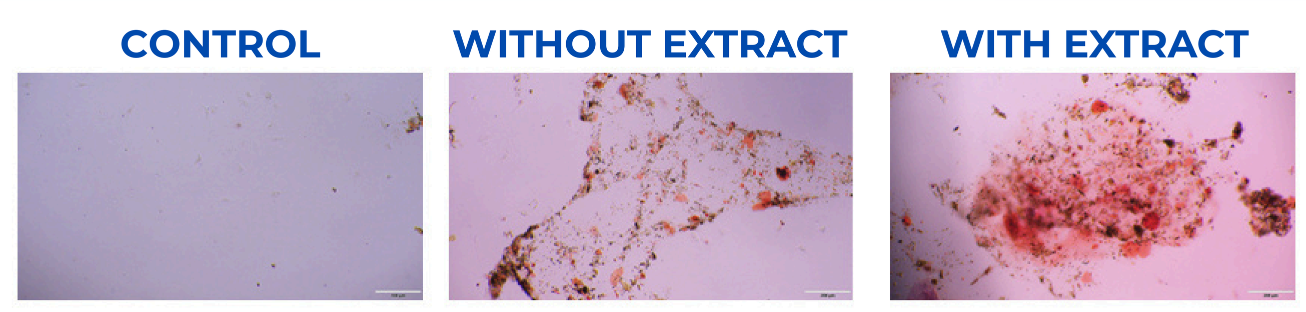


The hemostasis test results show the superior local hemostatic ability of the scaffold compared to commercial gauze.

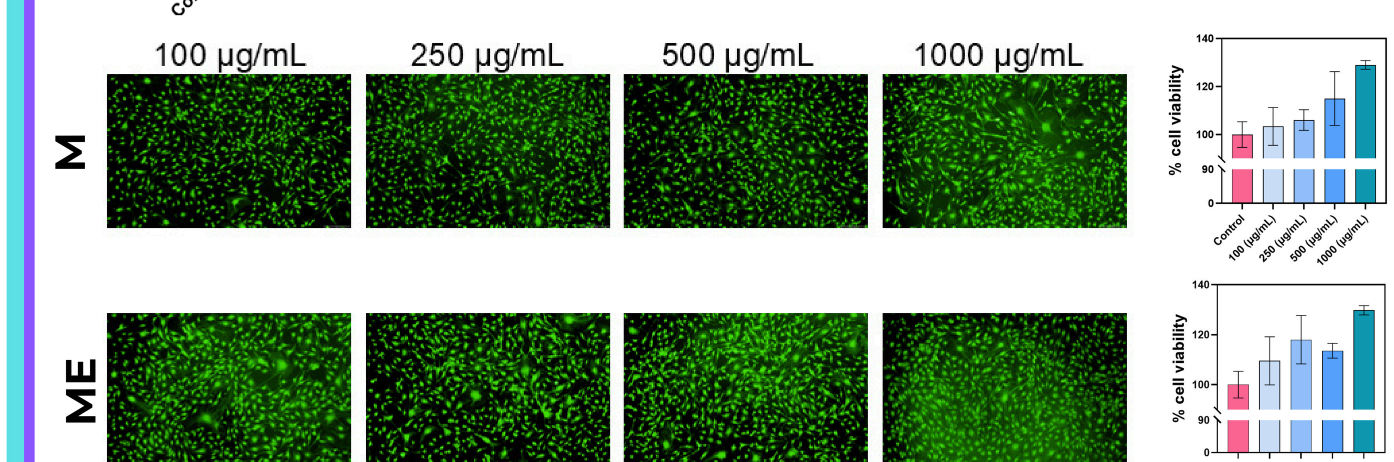


The hemolysis assay confirming their non-hemolytic nature and good hemocompatibility.





<u>**Alizarin Red S staining assay**</u>



<u>**MC3T3 Cell viability and proliferation**</u>

In summary, the mineralized scaffold **exhibited high cytocompatibility**, promoted *MC3T3 cell viability and proliferation*, and **enhanced osteogenic differentiation** as evidenced by *Alizarin Red S staining*

A scaffold was fabricated via thermogelation and **freeze-drying** and subsequently **immersed** in fivefold concentrated **SBF (SBFx5)** supplemented with ***Cissus quadrangularis*** extract to enhance bioactivity, as confirmed by FTIR and XRD analyses. The scaffold exhibited high porosity with an interconnected pore network conducive to cell infiltration, and biocompatibility assessments revealed no adverse effects. <u>These findings highlight the potential of integrating natural polymers with mineralization and plant extracts</u> to develop **eco-friendly, bioactive scaffolds** for **bone tissue engineering**.

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[2] Hoveidaei, A. H., Sadat-Shojai, M., Nabavizadeh, S. S., Niakan, R., Shirinezhad, A., Mosalamiaghili, S., & Tabaie, S. (2025). Clinical challenges in bone tissue engineering-A narrative review. Bone, 192, 117363.
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