Research Summary

Customized Combinatorial Approaches in Regenerative Bone tissue engineering

for the Treatment of Bone loss and Bone diseases

Prof. Sailaja's Research in Bone tissue engineering is focused on design and development of diverse regenerative scaffold systems with ***multi functionalities*** *to aid faster/sequential osteogenesis for the treatment of bone diseases such as* ***osteosarcoma****,* ***osteomyelitis*** *etc. in addition to* ***normal bone-grafting.*** *These bone regenerative tissue engineering approaches could be classified mainly into five categories.*

1. *Novel surface phosphorylation protocol to enable biomimetic and biomineralization of diverse biomaterials that promote bone-bonding and new bone formation in vivo*

2. *Advancements in designing combinatorial systems for regenerative bone tissue engineering for osteosarcoma*

3. *Development of customizable and herbal hybrid BTE scaffolds with tunable degradation for bone implants such as screws and grafts*

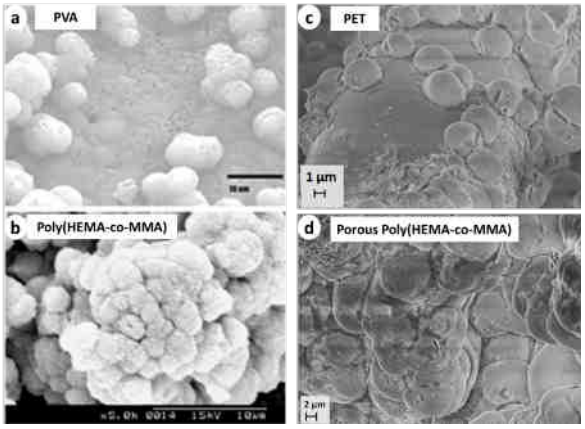
4. *Theranostic nanomaterials for treating Osteosarcoma (Bone Cancer)*

5. *Injectable materials in Bone Tissue Engineering*

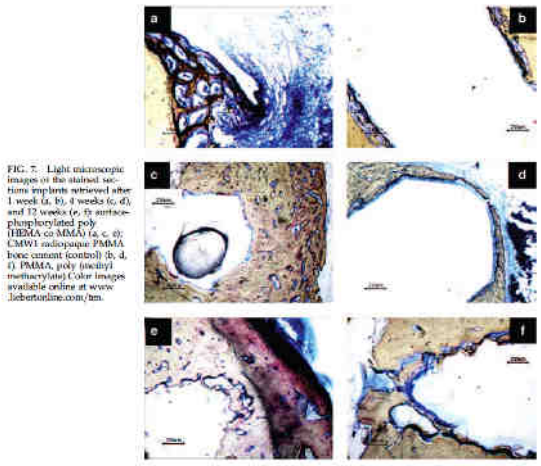
1. ***A novel surface phosphorylation protocol that enable biomimetic and biomineralization of diverse biomaterials and promote bone-bonding in vivo***

Millions of patients around undergo bone grafting/ surgical treatments annually. Commercially available PMMA based bone cement presents clinical challenges with respect to multiple types of osteolysis and implant failure due to non-osseointegration (non-bone bonding) between PMMA bone cement and host bone; hence fail to do load transfer and ultimately leading to implant failure. This work presents development of a novel surface phosphorylated copolymer of MMA and HEMA using a novel surface phosphorylation technique. Smart surface functionalization of the material essentially generate a biomimetic interface which would be cell-friendly, leading to better cell-material interactions favouring cell adhesion, anchorage, proliferation and signalling (Sailaja et al., 2016). Phosphorylation is an effective strategy to impart surface functionalization to scaffolds intended for bone regeneration. The ***simple, unique strategy of surface phosphorylation*** by phosphorous pentoxide and o-phosphoric acid that induces is a generic method that can be used for phosphorylating biomaterials containing –OH groups on its surface as such or when subjected to esterification and upon successful phosphorylation apatitic calcium phosphate would be nucleated in a biological milieu (biomineralization- in presence of cells) or simulated physiological conditions (biomimetic mineralization), a critical event in bone tissue formation. *Uniqueness of the phosphorylation protocol developed is mainly embraced by eliminating need of any toxic solvents such as DMF, DMSO, etc*. In addition, the strategy could be adopted to various natural as well as synthetic materials (either in bulk or porous form or as nanoparticles/structures) by modulating the conditions based on the substrate characteristics. Most importantly, it is applicable in a wide temperature range from room temperature to 80^{o}C. The surface phosphorylated copolymer developed in this study invoked remarkable ***new bone formation in vivo***, validated in rabbit model as per ISO 10993. This surface phosphorylation technique has been explored for surface functionalization of several biomaterial substrates to aid biomineralization and augment bone regeneration eg: Poly (hydroxyethyl-co-methyl methacrylate) [poly(HEMA-co-MMA]- solid and porous (Gopalakrishnanchettiyar et al., 2009; Sreeja et al., 2021) (ii) Polyethylene terephthalate (Sreeja et al., 2020), and (iv) Polyvinyl alcohol (Sailaja et al., 2009).

The key path ways associated with cellular interactions that leads to osteogeneis as a result of surface phosphorylation can indeed lead to strategic design of functional designing of bone regenerative biomaterials. Based on these findings, we have submitted a proposal to SERB, Government of India, to elucidate the signalling mechanism underlying surface phosphorylation-induced osteogenesis. The proposal has been accepted under the National Post–Doctoral Fellowship scheme – Basic Science in 2021 (File. No. PDF/2020/002685), and the work is in progress.



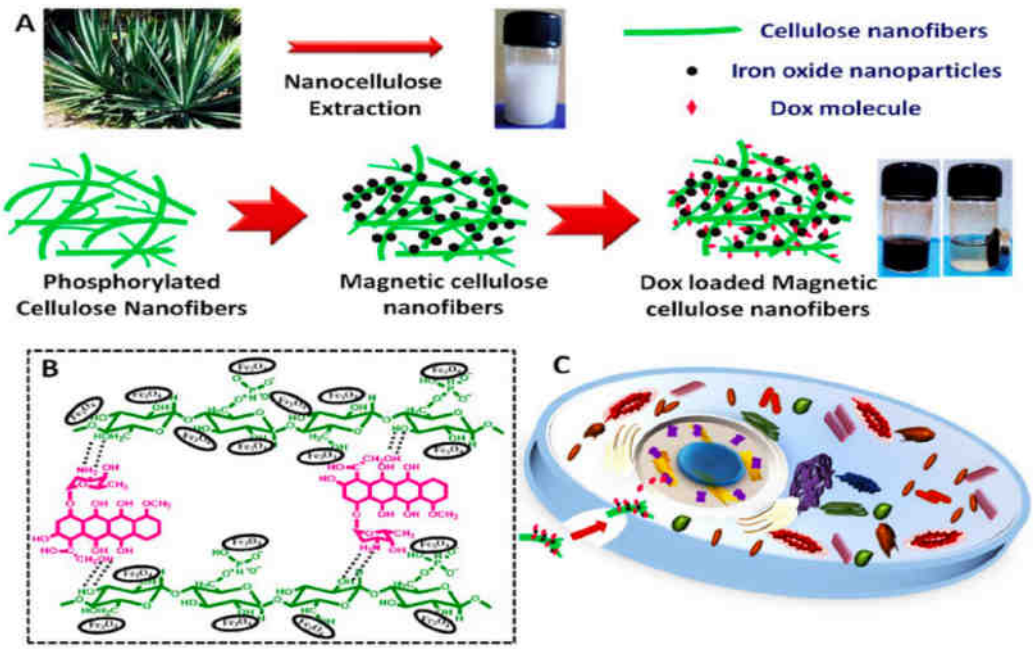
Biomimetic mineralization of Calcium Phosphate upon surface-phosphorylation: Different substrates[2–5]



New bone formation: one, four and twelve weeks when surface phosphorylated poly(HEMA-co-MMA implanted in Rabbit *Ref: (Gopalakrishnanchettiyar et al., 2009)*

(b) Magnetically modified phosphorylated nanocellulose for targeted Cancer therapy

A novel dual functional nano platform is synthesized from phosphorylated nanocellulose and superparamagnetic iron oxide nanoparticles (SPIONs) that combines magnetic hyperthermia and targeted drug delivery aspects to enhance the efficacy and to reduce the side effects of non-targeted chemotherapeutic drug delivery in cancer is developed (Sumitha et al., 2021). It is pH responsive so that at drug release happens preferably at pH, analogous tumour microenvironment. The targeted chemotherapeutic drug release, increased site-specific bioavailability, effective magnetic hyperthermia potential and pH-responsive controlled release of Doxorubicin (Dox) have been accomplished with a single system.[6]



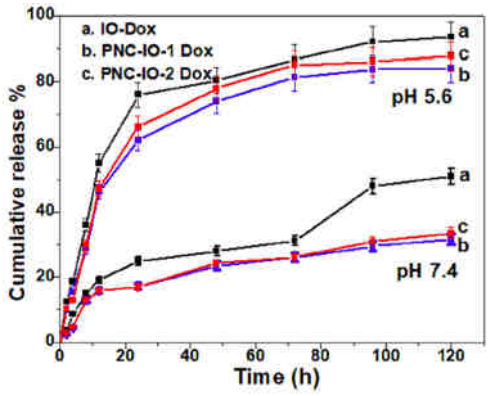


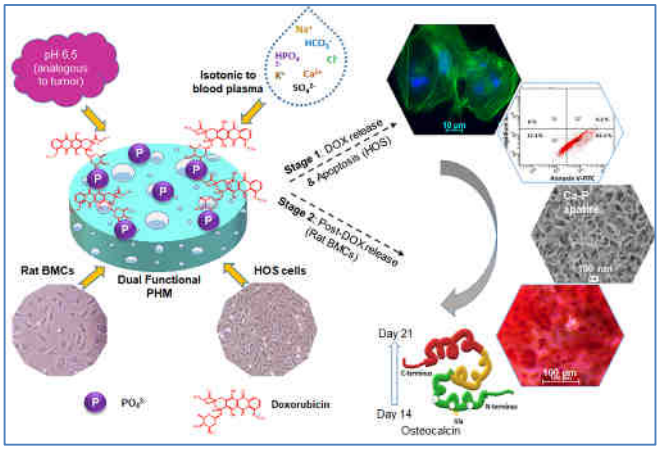
Fig. 9. Drug release profile of IO Dox, PNC-IO-1 Dox and PNC-IO-2 Dox at pH 5.6 (acetate buffer) and pH 7.4 (PBS), respectively, up to 120 h.

(Sumitha et al., 2021)

2. *Advancements in designing combinatorial systems for regenerative bone tissue engineering*

(a) comprehensive therapeutic approach for osteosarcoma

A bi-front therapeutic approach for osteosarcoma is presented this study. Surface-phosphorylated hierarchically porous hydrophilic therapeutic scaffold [Doxorubicin-loaded poly(HEMA-co-MMA)] was developed to present a comprehensive therapeutic approach for osteosarcoma that performs two active roles - pH-responsive sustained chemotherapeutic drug release followed by inherent bone formation and (*kindly see the schematic representation below*). In addition, it also induces apoptosis of Human Osteosarcoma (HOS) cells by establishing drug delivery and thereby tumor cell annihilation. Most importantly, inducing biomimetic mineralization successive to sustained DOX-release emphasizes its functionality as a bi-faceted treatment system for osteosarcoma. Hence, the scaffold presented would be a promising therapeutic strategy against osteosarcoma by the localized chemotherapeutic drug delivery subsequently induction of bone regeneration in a customized way.



Schematic illustration depicting the functionality of surface-functionalized poly(HEMA-co-MMA)

(Sreeja et al., 2021)

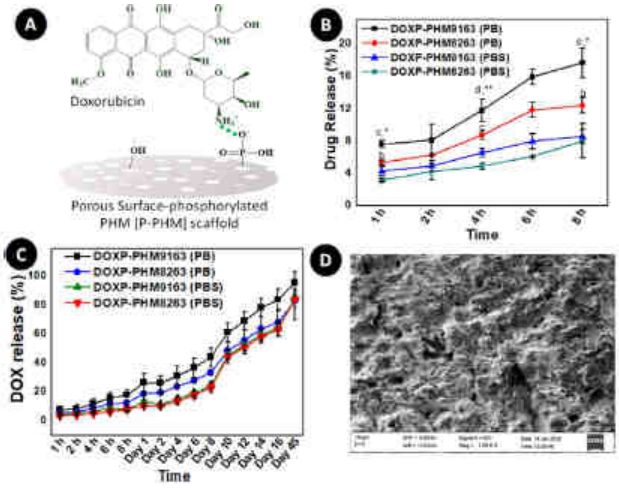


Figure 10. Cumulative release profile of DOX from DOXP-PHM9163 and DOXP-PHM8263 in PB (pH 6.5) and PBS (pH 7.34). (A) Schematic presentation of possible interaction between the phosphorylated scaffold and DOX; (B) DOX release for short period of time; (C) DOX release for prolonged period of 45 days; (D) FESEM image depicting surface morphology of DOXP-PHM9163 after DOX release for 45 days (scale bar is 10 μm). Note: all values are presented as mean ± SD. "b" and "*", "c" and "**", and "d" indicate statistical significance ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively) compared with DOXP-PHM9163 (PBS) and DOXP-PHM8263 (PB).

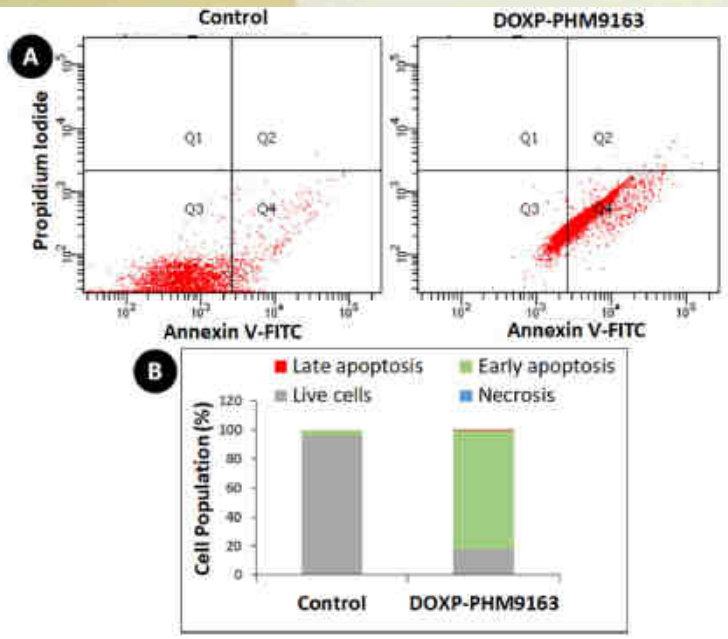


Figure 12. Annexin V/PI double staining of HOS cells treated with the extract of DOXP-PHM9163 for 24 h. (A) Representative scatter plots of FACS analysis following treatment with DOX released from DOXP-PHM9163. [(Q_1) necrotic cells—annexin V^+/PI^+; (Q_2) late apoptotic cells—annexin V^+/PI^+; (Q_3) live cells—annexin V^-/PI^-; (Q_4) early apoptotic cells—annexin V^+/PI^-]. (B) Percentage cell population in each quadrant.

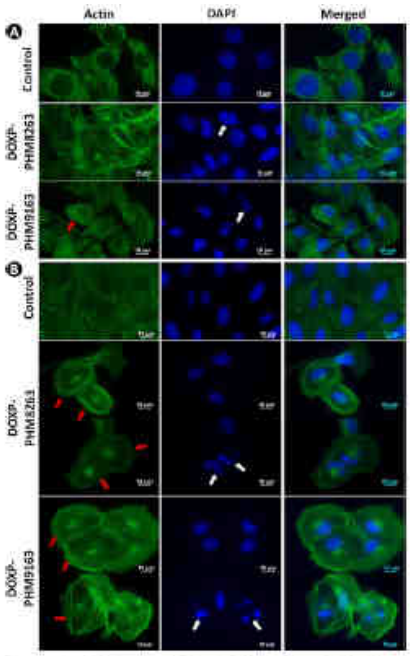


Figure 11. DOX released from DOXP-PHM scaffold induced alterations in actin cytoskeleton and nuclei of HOS cells. (A,B) Representative images of F-actin (green) and DAPI (blue)-stained cells treated for 24 and 48 h respectively, where white arrows highlight fragmented nuclei and red arrows indicate cortical ring formation. Scale bar is 10 μm.

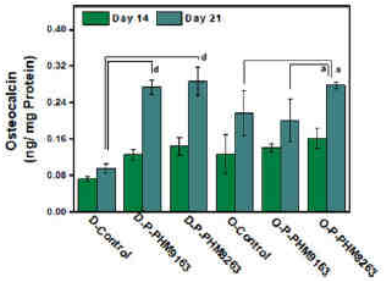


Figure 9. Osteoblast differentiation potential of the scaffolds—osteocalcin level [note: D-control, D-P-PHM9163 and D-P-PHM8263 indicate the treatment groups were supplemented with DMEM, while O-control, O-P-PHM9163, and O-P-PHM8263 represent the treatment groups supplemented with osteogenic medium. Note: all values are presented as mean ± SD. "a" indicates statistical nonsignificance ($p > 0.05$) and "d" represents statistical significance ($p < 0.001$)].

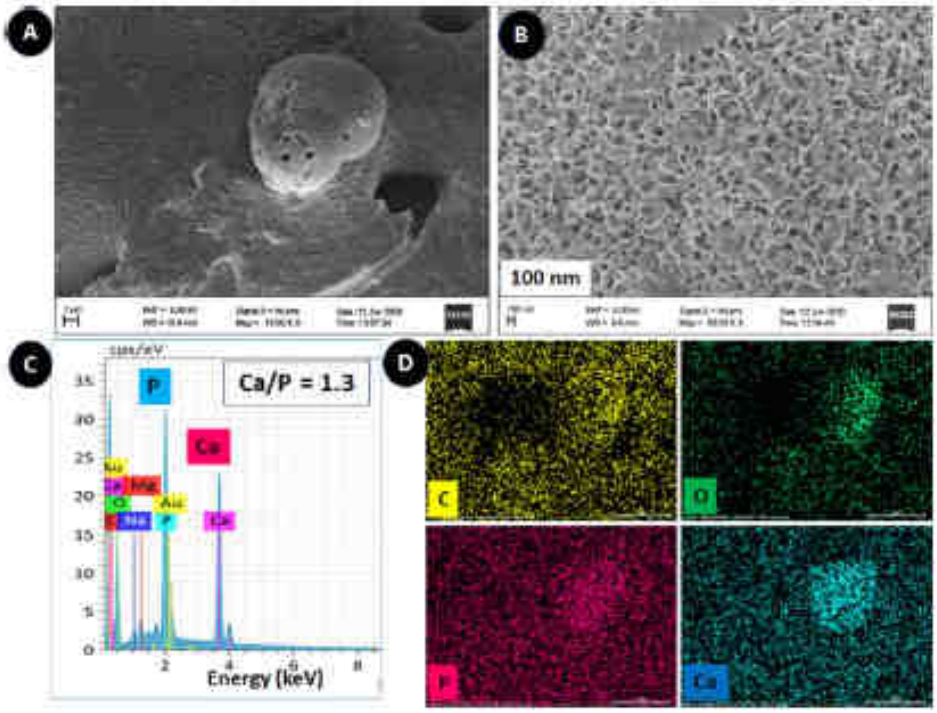
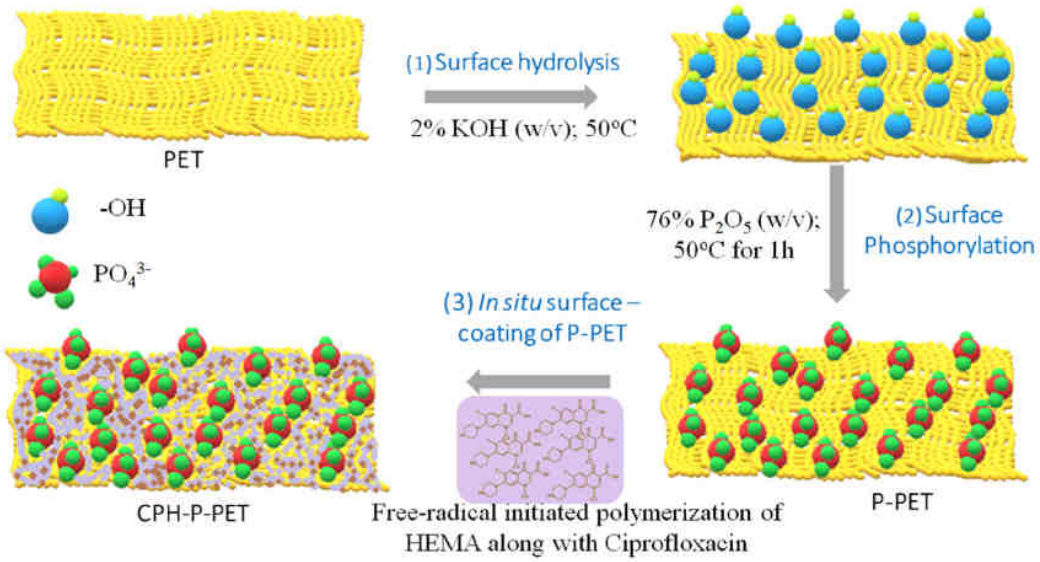


Figure 13. Proof of post-release biomimetic mineralisation of scaffolds after sustained DOX release for 45 days. [(A,B) FESEM images depicting intense Ca-P coating (two different magnifications); (C,D) corresponding EDX spectrum and elemental mapping].

(b): Comprehensive therapeutic approach for osteomyelitis

The surface-phosphorylated wet-spun PET fibrous matrix coated with ciprofloxacin-impregnated biodegradable polymer, serves as a dual therapeutic scaffold that exhibited high bactericidal activity and osteogenic potential.The dual functional surface-transformed PET scaffold [Ciprofloxacin-impregnated HEMA coated phosphorylated PET (CPH-P-PET)] was designed to address bone infection and provide a platform for repairing and regenerating damaged bone (*kindly refer to the schematic representation below*). The CPH-P-PET also served as an excellent antibiotic delivery vehicle based on the cumulative release profile of ciprofloxacin. *In vitro* calcium phosphate apatite formation and other biofunctional assays depict its proficiency in new bone formation. Furthermore, confirmative evidence from the expression of osteogenic biomarkers – Alkaline phosphatase (ALP) and Osteocalcin (OSN) - in rat Bone marrow Mesenchymal Cells (BMCs) elucidated the osteoinductive nature of the scaffold. Altogether, the antibiotic carrying surface-transformed PET is an excellent dual-functional scaffold for bone regeneration and prohibits bone-specific infections.



Scheme 1. Fabrication of dual functioning CPH-P-PET system.

Schematic illustration of surface-transformed dual-functional PET

(Sreeja et al., 2020)

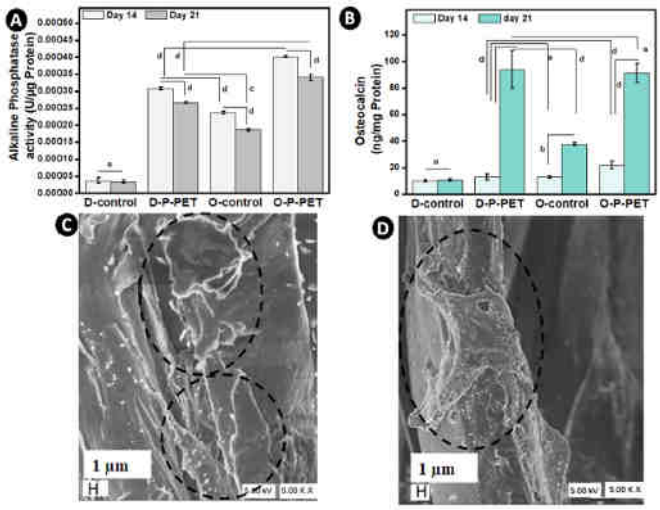


Fig. 9. Levels of osteogenic differentiation markers – alkaline phosphatase and osteocalcin - in bone marrow mesenchymal cells. Specific activity of ALP in BMCs cultured in both normal and osteogenic medium is expressed as U/µg protein (A). The amount of OSN is expressed in ng/mg protein (B). [D-control: BMC alone in DMEM; D-P-PET: BMC cultured on P-PET supplemented with DMEM; O-control: BMC cultured in ODM and O-P-PET: BMC cultured on P-PET in ODM]. C and D: FESEM of BMCs on P-PET on day 14. Note: All values are presented as mean ± SD. 'a' indicates non-significance ($p > 0.05$); 'b', 'c' and 'd' indicates statistical significance with p values < 0.05, < 0.01 and < 0.001, respectively.

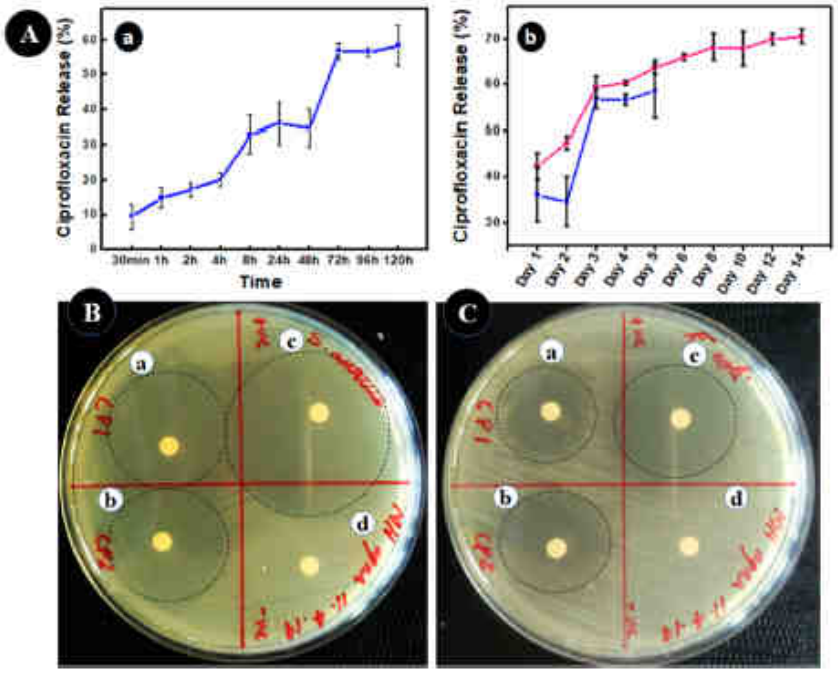


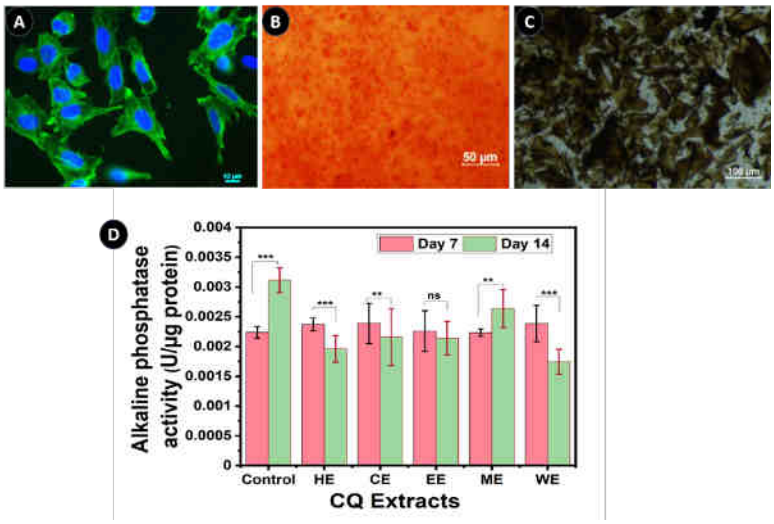
Fig. 10. Time-dependent antibiotic – release from CPH-P-PET [A(a): short-term release for 5 days; A(b): combined graph of drug release for 5 days (blue line) and two weeks (pink line)] and inhibition of bacterial growth [S. aureus (B) and E. coli (C)] on agar plates. B(a) represents extract collected at 8 h and B(b) represents extract collected at 24 h. Positive control B(c) is ciprofloxacin (3 µg/20 µl.) and negative control B(d) is Milli-Q water. Note: all values are presented as mean ± SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Knowledge about the cellular interactions on surface functionalized polymers can facilitate the strategic design of functional biomaterials. Based on this thought, we have submitted a proposal to Science Engineering Research Board, Government of India, to elucidate the signalling mechanism underlying surface phosphorylation-induced osteogenesis. The proposal has been accepted under the ***National Post–Doctoral Fellowship scheme (File. No. PDF/2020/002685) 2021 Basic Science*** *and the work is in progress. We anticipate that the research outcome would certainly be effective for developing an osteogenic platform for Regenerative BTE*

3. Customizable and herbal hybrid scaffolds with tunable degradation for Bone grafting

(a) Identification of osteogenic competency of Cissus quadrangularis Linn stem extract

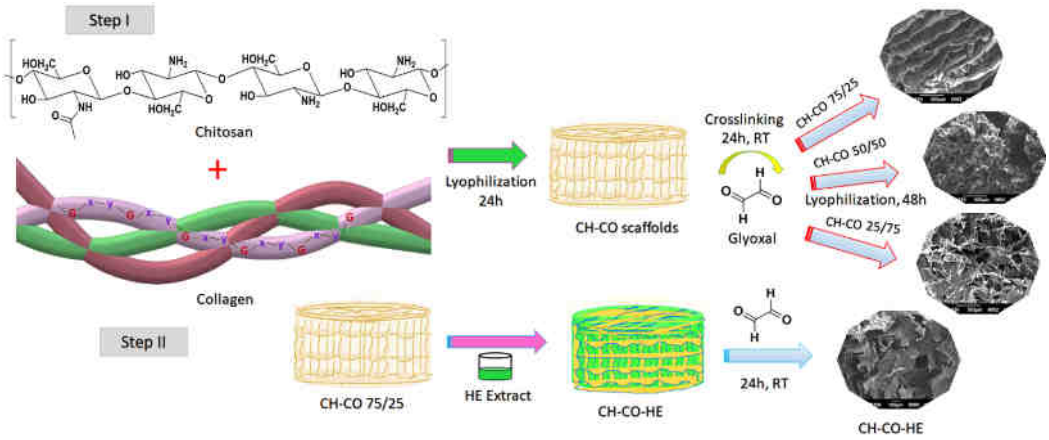
Contemporary demand calls for a high restorative index as an indispensable requirement for bone tissue engineering scaffolds, where therapeutic agents of natural origin function as a modulator for new bone formation become of utmost importance. This study systematically investigated the edible stem part of *Cissus quadrangularis* (CQ) *Linn* as a natural resource of bioactive metabolites capable of invoking early biomineralization and osteogenesis. Sequential extraction of CQ stem . (n-hexane\chloroform\ethyl acetate\methanol\water). Phytochemical screening: hexane extract (HE), chloroform extract (CE), ethyl acetate extract (EE), methanol extract (ME) and aqueous extract (WE) by qualitative and quantitative assays, UV-Visible and FTIR spectroscopic analyses. Cytocompatibility and proliferation index, effect on cellular architecture: MTT assay and F-actin/DAPI staining in Human Osteosarcoma (HOS) cells. WE and HE were found to have significant osteogenic potential from the expression of alkaline phosphatase as early as 7 days in cells treated with WE and HE. Configuring abundance of bone regenerative phytochemicals in HE and WE, this work in fact present ample opportunities for customized bone tissue engineering and development of a completely bioresorbable bone regenerative scaffold of natural origin.



Cytocompatibility and biofunctional evaluation of CQ extract. A, B and C represents cytoskeleton staining, ARS and von-kossa staining of HE extracts whereas D represent Alkaline phosphatase activity of HE extracts in HOS cells. (P. R. Nair et al., 2021)

(b) Completely biodegradable herbal Scaffold from CQ for regenerative bone tissue engineering

A bio-inspired natural chitosan-collagen hybrid (CH-CO) scaffold integrated with CQ stem hexane extract (HE) capable of invoking early biomineralization and thereby hasten bone regeneration efficacy has been developed. The mechanically empowered chitosan-collagen porous scaffold with a controlled degradation profile is fabricated using a biocompatible crosslinking agent, glyoxal, wherein the amino groups of chitosan and collagen are effectively utilized for establishing a cytocompatible crosslinking with glyoxal. Sequential formulations of chitosan-collagen scaffolds (CH-CO 75/25, CH-CO 50/50, CH-CO 25/75) were prepared by freeze-drying to obtain porous structure resembling native bone, followed by crosslinking with glyoxal to tune biodegradation profile. Physico-chemical, mechanical, and morphological characterization of scaffolds was performed, followed by swelling and degradation analysis, and the best composition with the highest mechanical properties with favourable degradation kinetics has been identified. This hybrid CH-CO scaffold was formulated and integrating HE in this composition, and its bio-efficacy has been validated by matrix biomineralization and matrix-cell interaction assays.



Scheme. Schematic illustration of the Fabrication of CH-CO and CH-CO-HE **(P. Nair et al., 2021)**

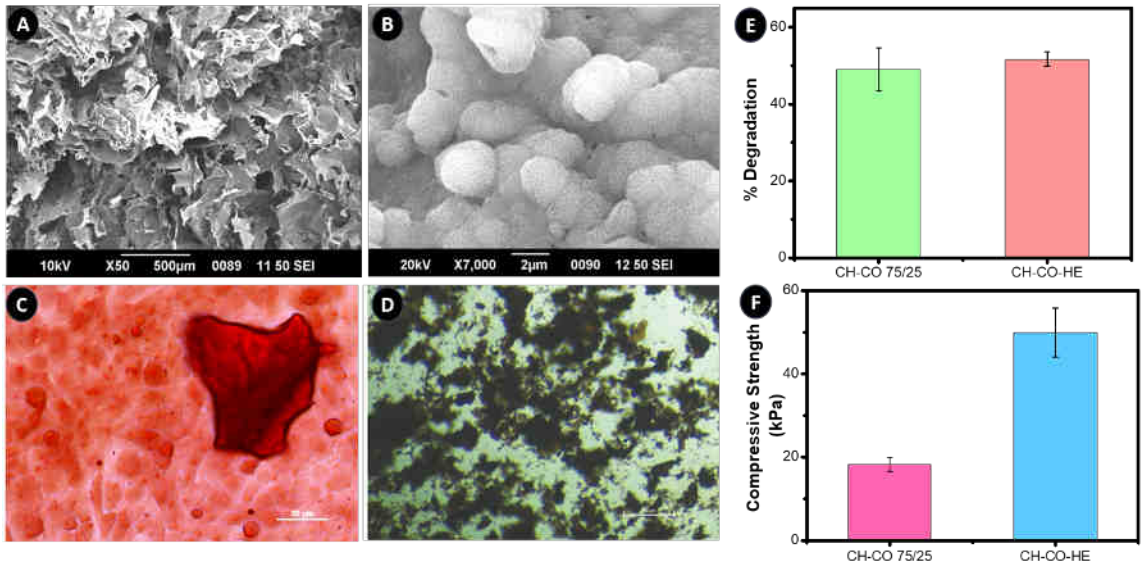


Figure A and B represents SEM images of bare CH-CO-HE scaffolds and mineralized CH-CO-HE scaffolds. Whereas C and D represents ARS and von-kossa staining of CH-CO-HE scaffolds after 7 days of incubation in HOS cells. D and F are the degradation profile and compressive strength of CH-CO-HE scaffolds.

(c) *Cissus quadrangularis Linn* stem fiber based herbal scaffold for accelerated bone healing

We have discovered for the first time the unique intrinsic biomineralization and bone regeneration of CQ stem fibres as a competing biofunctional substrate capable of eliciting biomineralization under *in vitro* conditions. CQF is indigenously strong on account of its longitudinally organized microfibrous array of cellulose that set forth a slow degradation kinetics as well. It is inherently cytocompatibile and elicits very early biomineralization at 72h with Ca/P ratio near to that of human bone apatite while osteogenic competency was validated by estimating alkaline phosphatase (ALP) activity in rat Bone marrow Mesenchymal Cells (BMC) and the results of the study manifest CQF as a potential BTE substrate with intrinsic osteoinductive functionality

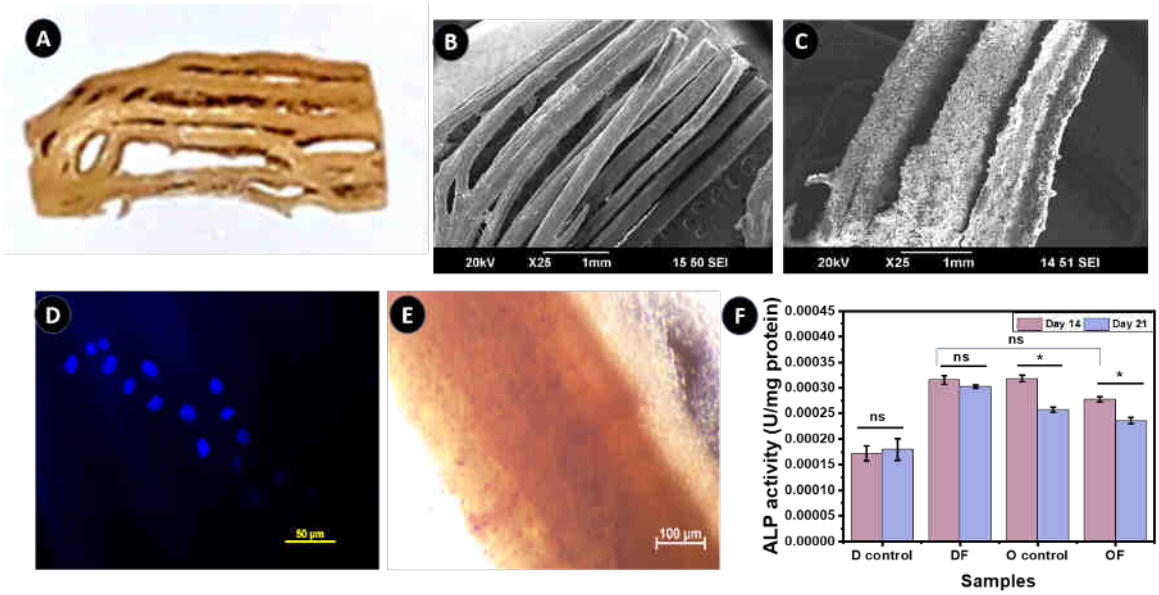
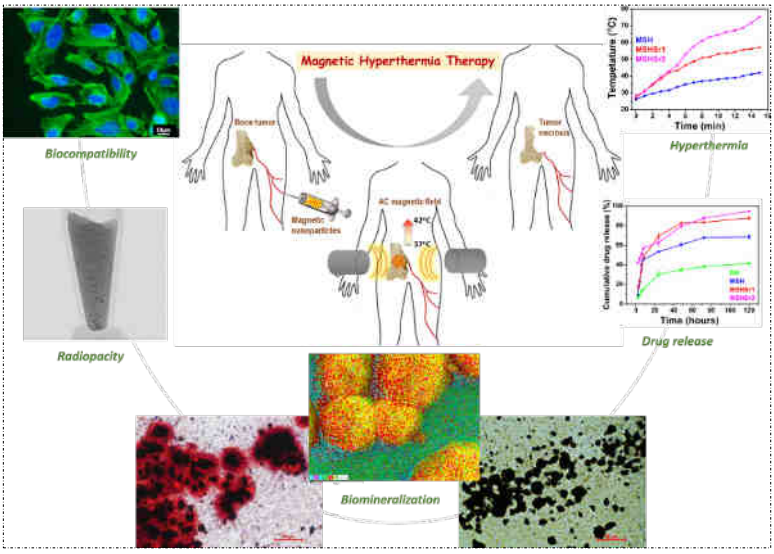


Figure Represent photographic image of CQF. B and C represents SEM images of CQF and mineralized CQF after 7 days of incubation in 1.5 X SBF. D and E are the DAPI staining and ARS staining images of CQF after incubation with HOS cells and F represent Alkaline phosphatase activity of CQF in BMC's at two different time points.

(P. Nair et al., 2021)

4. Theranostic materials for Osteosarcoma

Osteosarcoma is the most common primary **bone cancer** occurring in children as well as adolescents. Contemporary treatment modalities like surgical tumour resection, hormone therapy, chemotherapy and radiotherapy are inefficient in improving the survival rate of osteosarcoma patients. Superior alternatives like hyperthermia, photodynamic therapy and low/high-intensity pulsed ultrasound sonodynamic therapy exhibited enhanced cure rates and hence they are in the limelight recently. In magnetic hyperthermia therapy for the eradication of deep-seated bone tumours, Superparamagnetic iron oxide nanoparticles (SPIONs) are magnetically targeted to tumour site and after cells uptake these nanoparticles, generate intracellular heat (temperature of 42 ◦C– 45 ◦C) upon induction of alternating magnetic field which is sufficient to induce tumour necrosis, locally keeping the healthy normal cells/tissue intact. when therapeutic materials are having imaging competencies they are termed as 'theranostic' materials; which completely eliminate the need of two separate systems to fulfil two distinct objectives of therapy and imaging. 'Nanotheranostics' is specific to the development of nanomaterials possessing theranostic potential. The widely used diagnostic methods in nanotheranostics include Computed tomography (CT), Magnetic Resonance Imaging (MRI), Positron emission tomography (PET), Single photon emission computed tomography (SPECT), Optical imaging etc.



Intrinsically radiopaque magnetic nanocomposite for combinatorial treatment of osteosarcoma

(Sneha, Sreeja, et al., 2021)

In this work, a multifunctional magnetic nanocomposite composed of maghemite, strontium doped hydroxyapatite and silica nanoparticles prospectively holding indispensable therapeutic features such as ***magnetic hyperthermia, in vitro biomineralization, sustained drug release and intrinsic radiopacity*** is developed for the treatment of osteosarcoma by conventional sol-gel technique. MSHSr1 (the best composition) exhibited a ***saturation magnetization of 47.4 emu*** g^{-1} and attained ***hyperthermia temperature (42*** ^{o}***C) at a very low exposure time of 4 min***. MSHSr1 is further unique with respect to its exceptional x-ray attenuation ability (***contrast enhancement 154.5% in digital radiography; CT number 3100 HU), early biomimetic mineralization*** (*in vitro*) evident by the formation of spheroidal apatite layer (***Ca/P ratio 1.33***) harvested from FESEM–EDX analysis and controlled release of ***Doxorubicin, the clinically used chemotherapeutic drug: 87.7% at 120 h*** in tumour analogous **pH (6.5)** when compared to **physiological pH (71.3% at 7.4)**. MTT assay complemented with cytoskeleton (F-actin) staining of human osteosarcoma (HOS) cells affirm biocompatibility of MSHSr1**.** ***In vitro biomineralization authenticated by Alizarin red S and von Kossa staining*** has been further corroborated by semi-quantitative calcium estimation of HOS cells cultured with MSHSr1 for two weeks. The results therefore validate the multifunctionality of MSHSr1 as a combinatorial therapeutic nanocomposite for osteosarcoma treatment.

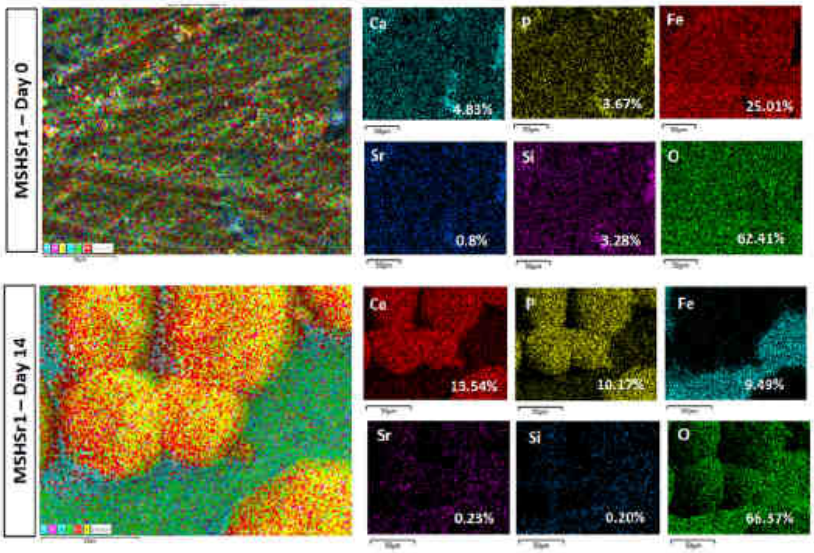


Figure 6. Elemental mapping of MSHSr1 pellet before and after immersion in SBF for 14 d.

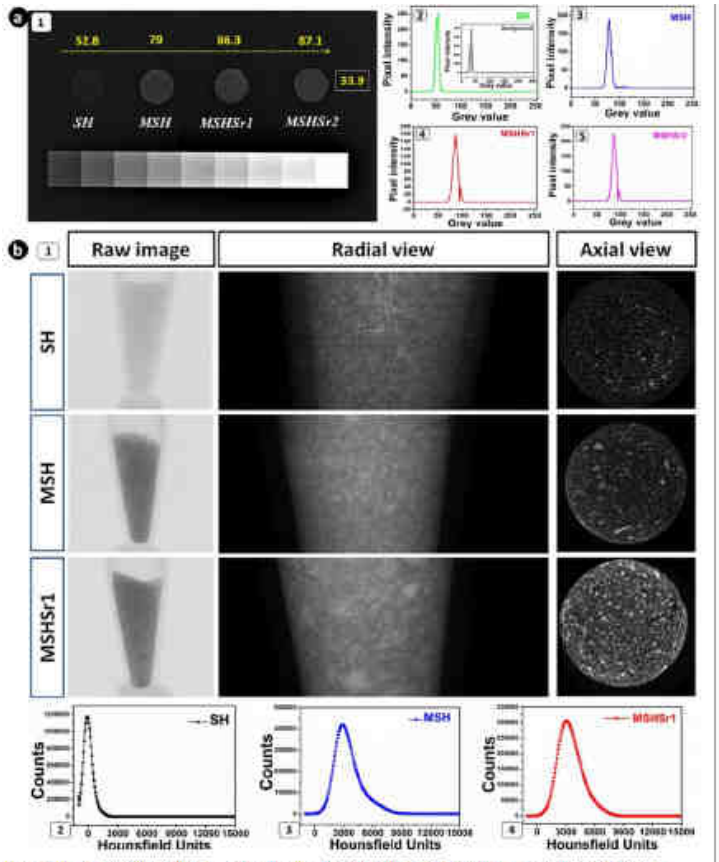


Figure 7. Digital x-ray radiographic images of nanocomposite pellets with standard aluminium step wedge (a1), histogram profiles of grey scale distribution of different nanocomposites (a2)–(a5), micro-CT images (raw images, radial and axial view of 2D reconstructed images) of sample powders placed in micro centrifuge tubes (b1) and their respective HU histogram (b2)–(b4).

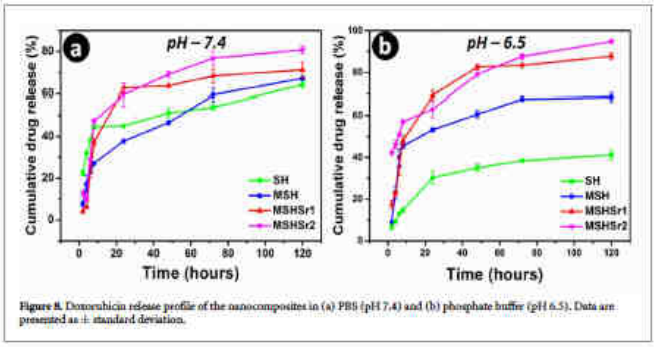


Figure 8. Doxorubicin release profile of the nanocomposites in (a) PBS (pH 7.4) and (b) phosphate buffer (pH 6.5). Data are presented as ± standard deviation.

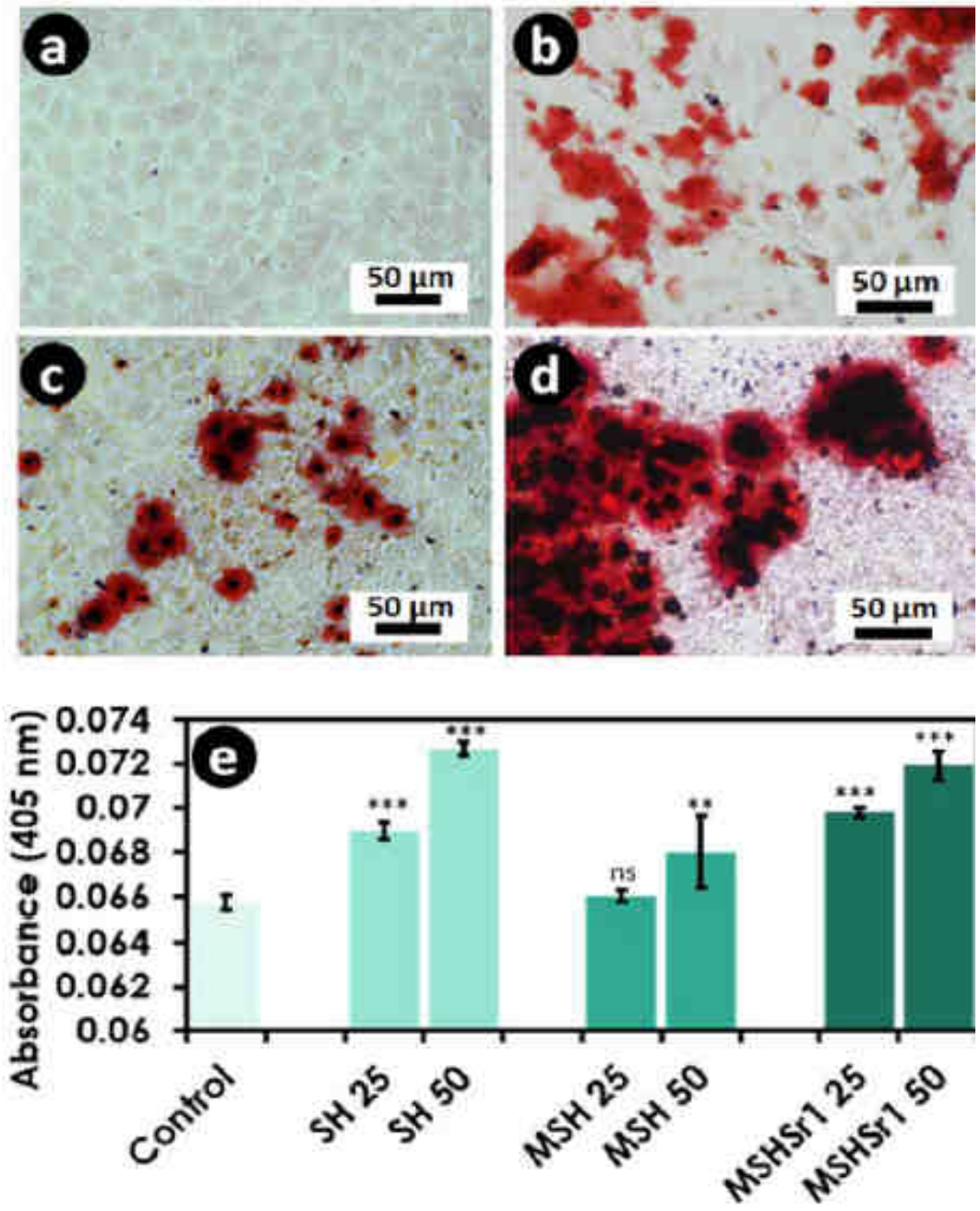
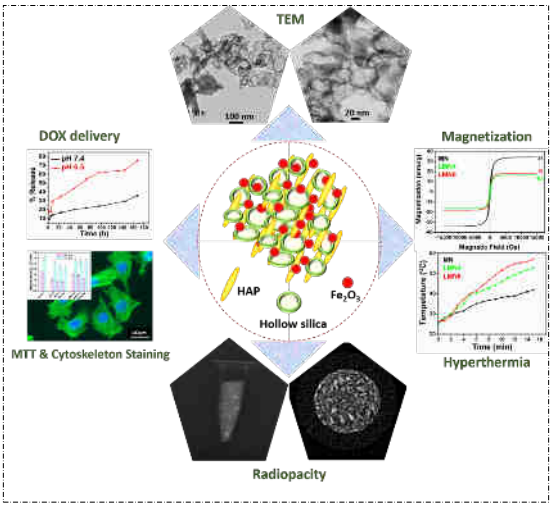


Figure 11. ARS-stained images of (a) control (b) SH, (c) MSH (d) MSHSr1 and (e) semi-quantification of Ca deposits by ARS staining. ns refers to $P > 0.05$, ** refers to $P < 0.01$ and *** refers to $P < 0.001$ vs control.



Natural Rubber Latex assisted Shape Attuned synthesis of Magnetic nanocomposite endowing X-ray visibility for cancer therapeutics

(Sneha, Benny, et al., 2021)

Shape-attuned design plays a remarkable role in modulating the hyperthermia potential of magnetic nanomaterials intended for targeted cancer therapy. Accordingly, a customized approach has been adopted to synthesize a magnetic bioceramic nanocomposite comprising maghemite, hydroxyapatite (HAP) and silica nanoparticles by the sol–gel method, wherein biosynthesized natural rubber latex served as a colloidal sacrificial hard template to modulate its ultrastructure via a controlled sintering process and exhibited a comprehensive morphology consisting of hollow silica quasi-nanospheres adjoined to hydroxyapatite nanorods. The nanocomposite has high hyperthermia potential (42 ^{o}C in 6 min). Digital X-ray radiography proved its attenuation efficacy with a grey value of 113 and a contrast enhancement of 121.5% compared with its adjacent black background, while micro-CT analysis validated the radiopacity of LMN6 with a CT number of 1353 HU. Moreover, the system offered a favourable drug loading efficiency (60.5%) with a sustained and targeted release of 76% of the chemotherapeutic drug, ‘doxorubicin’ at the slightly acidic tumour pH of 6.5 within 7 days. Besides, MTT assay and F-actin staining authenticate its biocompatibility, presenting this biomedically relevant system for its utility in combinatorial cancer therapy.

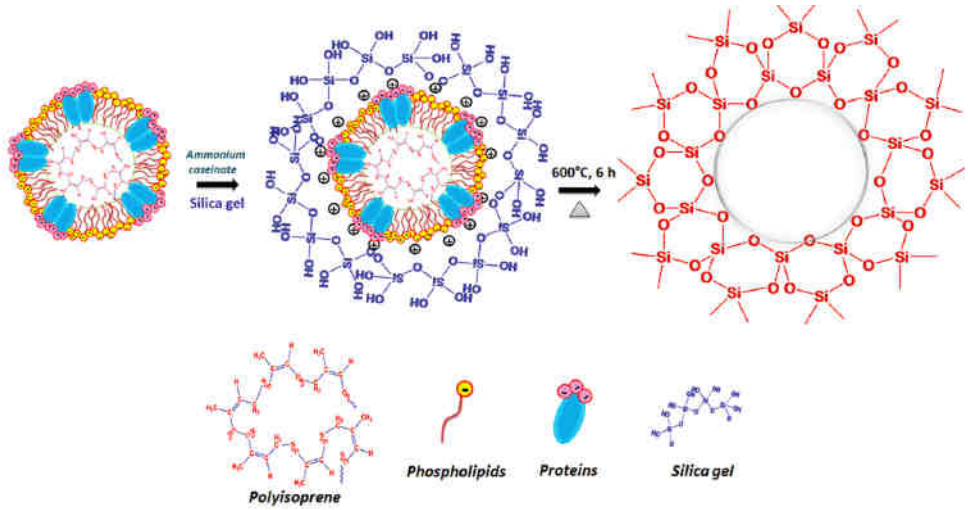
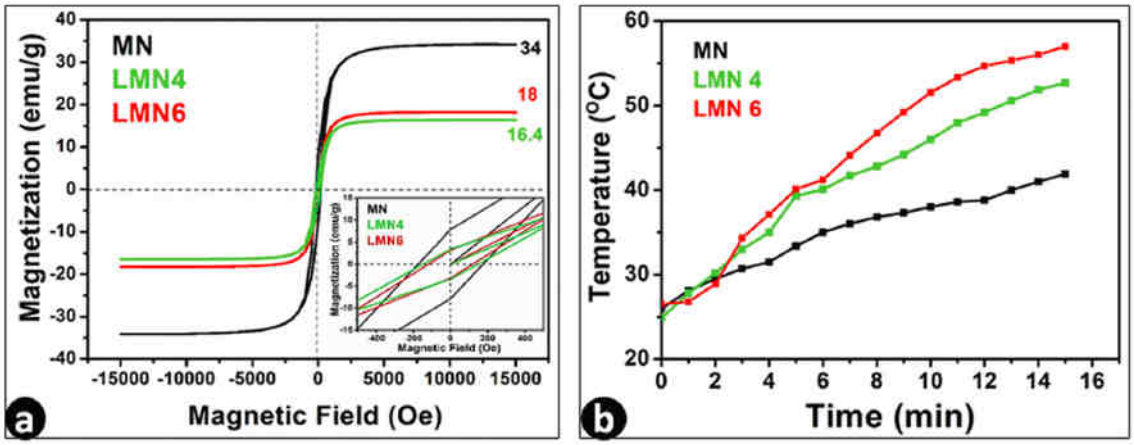


Fig. 5 Schematics of the formation of hollow silica nanospheres assisted by NRL hard templates.



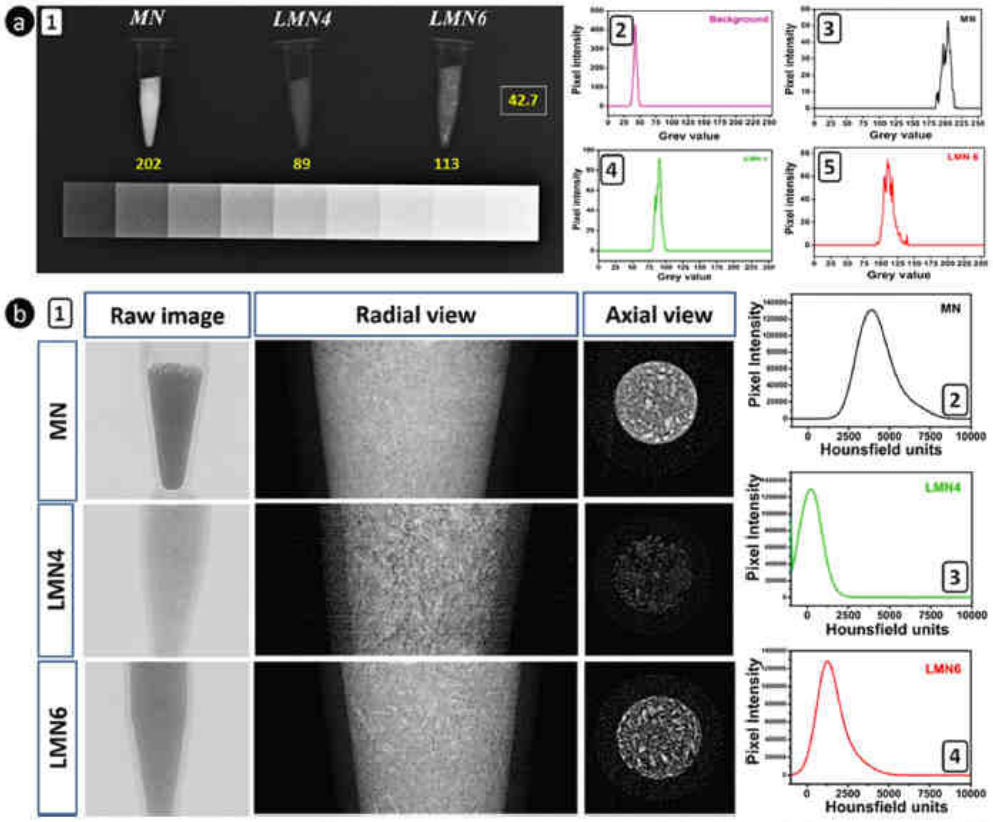


Fig. 8 Digital X-ray radiographic greyscale image (a1), histogram profile of greyscale distribution (a2–a5), Micro-CT 2D greyscale images (b1) and HU histogram of MN, LMN4 and LMN6 (b2–b4).

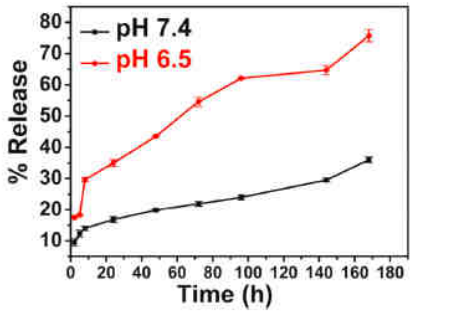
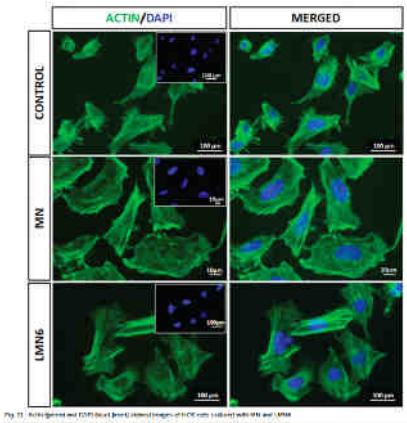


Fig. 9 *In vitro* DOX release behaviour of LMN6 at physiological pH (7.4) and at the slightly acidic tumour pH (6.5).



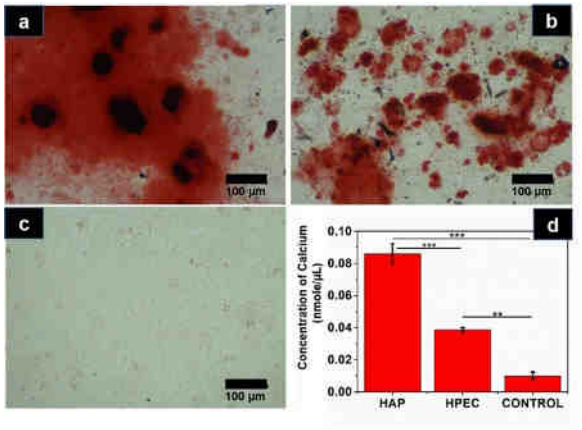
5. Injectable Osteoinductive materials for osteomyelitis

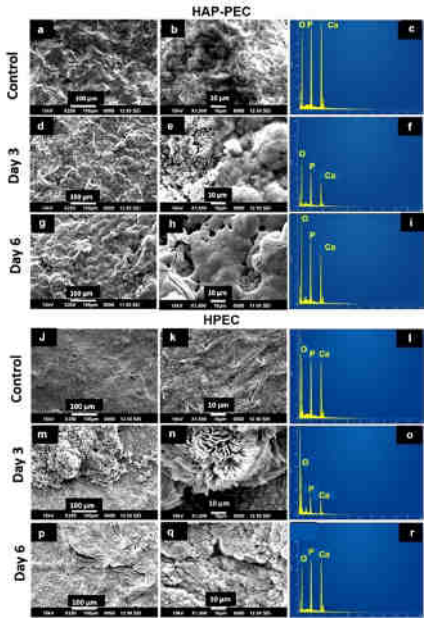
Osteomyelitis, a bacterial infection that affects bone-marrow, periosteum, cortex and associated soft tissues lead to inflammation which can be certain times detrimental. The extent of occurrence of this infectious diseases has been recently found increasing due to larger number of bone injuries and subsequent use of orthopaedic implants. In addition, increased incidence of diabetes mellitus, inadequate vascularization and other blood stream infections also contribute to osteomyelitis. The intensity of osteomyelitis depends on the onset pathway, immune system of the person and the microorganism responsible for the infection. Major challenges associated with osteomyelitis treatment are either poor biodegradability of the implanted systemic drug delivery device, creating requirement of an unavoidable surgery or inferior release profile of the antibiotic highlighting pivotal demand for alternative therapeutic options.

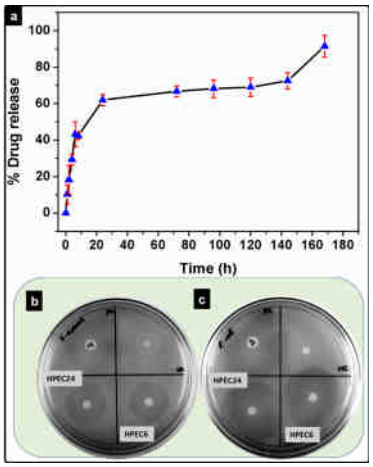
An injectable osteoconductive polyelectrolyte complex nanoconjugate capable of controlled delivery of ciprofloxacin has been developed by *in situ* conjugation of a novel biodegradable polyelectrolyte complex with antibiotic loaded nascent hydroxyapatite (n-HAP) for the treatment of osteomyelitis. Early biomimetic mineralization of apatite was manifested in simulated physiological condition with Ca/P =1.23 (day 3) and 1.55 (day 6) complimented by *in vitro* biomineralization of MG-63 and HOS cells in a week (Alizarin Red S staining) which was further validated by calcium quantification. Antibacterial efficacy of the injectable system against *S. aureus* and *E. coli* has been evaluated by delivery kinetics of ciprofloxacin and disc diffusion method. The injectable system therefore possesses unique combination of functionalities: osteoconduction enriched with early biomineralization, antibacterial and biodegradable; hence highly suitable for osteomyelitis treatment

Injectable Scaffold

(George et al., 2021)







Technology Development

(Indian Patent No: 202141026897, 16-06-2021- Porous Biodegradable Scaffolds for Regenerative Bone Tissue Engineering- G.S. Sailaja, Praseetha R. Nair, S. Sreeja)

Porous Biodegradable Scaffolds for Regenerative Bone Tissue Engineering.

This invention on 'Porous Biodegradable Scaffolds for Regenerative Bone Tissue Engineering' relies on developing a scaffold (matrix/substrate) system supportive of healing bone defects holding pronounced clinical relevance. The defects could be originated by trauma, surgical procedures or infections related to the bone. Generally, our body has the capacity to rectify bone defects by self-healing mechanism. However, when the defect dimensions exceed the critical size, self-healing potential of our body fails to regenerate the lost bone. In such a scenario, adequate support must be provided to augment bone defect repair. The traditional knowledge made us exposed to the leaves and roots of natural medicinal plants which are capable or repairing such bone damages and regenerate bone tissue. The tissue engineering scaffold we have designed is a herbal hybrid composite composed of microfibers of Cissus Quandrangularis (CQ) and a biodegradable clinically relevant polymer, polycaprolactone. The CQ fibres reframes the mechanical properties and impart a controlled degradation profile ensuring the sufficient retention for the scaffold until the defect is repaired and subsequently degrades. Such a synergetic combination would benefit regeneration phase by circumventing secondary surgical procedures that are being practiced generally to remove metal-based and other non-degradable implants after their intended service time in the body. Besides early biomineralization and expression of bone specific marker proteins, the porous architecture aids easy transport of nutrients and specific cells to towards the defect site and accelerate the tissue regeneration process. In short, this herbal scaffold delivers a native analogous environment to regenerate the bone through sequential cellular and molecular events, which undoubtedly presents a patient-friendly approach that minimizes secondary invasive procedures.

References

Gopalakrishnanchettiyar, S. S., Mohanty, M., Kumary, T. V., Valappil, M. P., Parameshwaran, R., & Varma, H. K. (2009). Surface-phosphorylated copolymer promotes direct bone bonding. *Tissue Engineering Part A*, *15*(10), 3061-3069.

George, L. H., A., A., S., P., & G.S., Sailaja (2021). Injectable polyelectrolyte complex nascent HAP nanoconjugate with Antibiotic Delivery potential for the treatment of osteomyelitis. *Biomedical Materials*, *Under Review*.

Nair, P., Sreeja, S., & Sailaja, G. S. (2021). Early biomineralizing chirosan-collagen hybrid scaffold with Cissus quadrangularis extract for regenerative bone tissue engineering. *New Journal of Chemistry*.

Nair, P. R., Sreeja, S., & Sailaja, G. S. (2021). In vitro biomineralization and osteogenesis of Cissus quadrangularis stem extracts: An osteogenic regulator for bone tissue engineering. *Journal of Biosciences*, *46*(4), 1-14.

Sailaja, G. S., Ramesh, P., Vellappally, S., Anil, S., & Varma, H. K. (2016). Biomimetic approaches with smart interfaces for bone regeneration. *Journal of biomedical science*, *23*(1), 1-13.

Sailaja, G. S., Sreenivasan, K., Yokogawa, Y., Kumary, T. V., & Varma, H. K. (2009). Bioinspired mineralization and cell adhesion on surface functionalized poly (vinyl alcohol) films. *Acta biomaterialia*, *5*(5), 1647-1655.

Sneha, K. R., Benny, N., Nair, B. N., & Sailaja, G. S. (2021). Natural rubber latex assisted shape-attuned synthesis of intrinsically radiopaque and magnetic bioceramic nanocomposite with hyperthermia potential for cancer therapeutics. *New Journal of Chemistry*.

Sneha, K. R., Sreeja, S., & Sailaja, G. S. (2021). Radiopacity endowed magnetic nanocomposite with hyperthermia and in vitro mineralization potential: a combinatorial therapeutic system for osteosarcoma. *Biomedical Materials*, *16*(4), 045029.

Sreeja, S., Muraleedharan, C. V., Varma, P. R. H., & Sailaja, G. S. (2020). Surface-transformed osteoinductive polyethylene terephthalate scaffold as a dual system for bone tissue regeneration with localized antibiotic delivery. *Materials Science and Engineering: C*, *109*, 110491.

Sreeja, S., Parameshwar, R., Varma, P. R. H., & Sailaja, G. S. (2021). Hierarchically Porous Osteoinductive Poly (hydroxyethyl methacrylate-co-methyl methacrylate) Scaffold with Sustained Doxorubicin Delivery for Consolidated Osteosarcoma Treatment and Bone Defect Repair. *ACS Biomaterials Science & Engineering*, *7*(2), 701-717.

Sumitha, N. S., Sreeja, S., Varghese, P. J. G., & Sailaja, G. S. (2021). A dual functional superparamagnetic system with pH-dependent drug release and hyperthermia potential for chemotherapeutic applications. *Materials Chemistry and Physics*, *273*, 125108.

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