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Curcumin loaded antibacterial eggshell – xanthan gum-based films an ingenious solution for biomedical application

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Abstract- **Current work uses agricultural waste and eggshells to convert them into biocompatible biomaterials. Eggshells can mimic the inorganic parts of natural bone (hydroxyapatite). Here, they are fortified with more versatile extracellular polysaccharides. H. Xanthan gum, combination. Another nano-formulation of curcumin was added to enhance the therapeutic antibacterial activity of the manufactured patch. Adhesive plasters showed optimized swelling rates after long-term treatment with SBF. The surface morphology of the manufactured patches was further characterized and analysed by XRD. Therefore, our data suggest that the patches produced may be a potential interdisciplinary approach for important wound healing applications**.

Keywords: **Hydroxyapatite, Egg shell, Xanthan gum, Films**

I.INTRODUCTION

Waste management in defined as recycling the discarded materials which has been an indigenous part of industries, household, hospitals and also agriculture [**1**]. Eggshell is typically considered to be a type of safe and non-hazardous agricultural waste, that can be used more sustainably [**2**]. Eggshells are also considered as waste and worthless without any transformation although this waste can increase the high risk of distribution of pathogens and malodours [**3**]. According to the values, the shell weighs about 11% of the egg mass and it constitutes of magnesium carbonate (1%), calcium carbonate (94%), organic matter (4%) along with calcium phosphate. By measurement, eggshell weight can be used as a biological prototype for catalysts, antimicrobial properties, cosmetics, and sources of calcium carbonate and biomaterials [5-6]. The main components of bone are the mineral phase (69%), organic matrix (22%), and water (9%) by weight. Hydroxyapatite is one of the steadiest and least soluble calcium phosphate bio ceramics with a stoichiometric Ca / p ratio of 1.67 [7-8]. Hydroxyapatite is widely used as an artificial bone substitute because of its related chemical properties as well as favourable biological properties including biocompatibility, bioactivity, biocompatibility, bone conduction, bone induction and bone integration. [9]. Because it easily binds to the apatite layer of bone, it is embedded in newly formed bone and hydroxyapatite that can be used in the low-calcium carbonate apatite layer of bone [10]. Curcuma longa belongs to the family Zingiberaceae and dates back to the ancient times of Ayurveda. Found primarily in tropical, subtropical and southeaster regions, it is a large growing area for use as a spice component [11-12]. Humans have a common practice of using medicinal natural resources. The composition of curcumin (C21H20O6), also known as diferuloylmethane, was first identified by Lampe and Milobedeska in 1991 [13]. Current research reports on the utilization of agricultural based wastes like eggshells and their conversion to biocompatible biomaterials for a variety of biomedical applications. Eggshells can mimic the inorganic parts of natural bone (hydroxyapatite). Here, they are fortified with more versatile extracellular polysaccharides. H. Xanthan gum, combination. Xanthan gum is a high molecular weight biopolymer with the ability to form physical and chemical networks, leading to its use in multiple drug delivery applications [14]. We added another nano-form of curcumin to increase the therapeutic potential of the resulting film and also analysed the antibacterial properties of the curcumin-loaded film. The film showed an optimized swelling rate during long-term treatment with SBF. [15]. Therefore, our data suggest that the films produced may be a potential interdisciplinary approach for biomedical applications.

II MATERIALS

Xanthan gum were obtained from HI Media. Egg shell waste were collected from local market. Acrylamide, Sodium Chloride, Acetic acid are the other chemical used in fabrication.

III METHOD

A. HOMOGENEOUS MIXTURE FOR FILMS FABRICATION

Eggshell waste was collected from a local market. The dishes were thoroughly washed with tap water, then distilled water, then NaCl solution. Eggshells are ground in a ball mill and finally sifted to a finer texture. The composite mixture of eggshell powder and XA was made in different weight ratios. In the next step, 0.5 g of the composite powder was mixed to the distilled water and mixed properly for at least 2 hours. The homogeneous mixture was heated at 70 ° C. in the presence of acetic acid and acrylamide with stirring. Finally, a curcumin-loaded formulation by Soumitra et al. The homogenized mixture was used to prepare a film on a glass slide and dried overnight. XRD analysis of the prepared eggshell XA film was performed on a Rigako Ultima IV diffractometer using Cu-Kα radiation.

B. ANTIMICROBIAL ACTIVITY OF FABRICATED FILMS BY AGAR DIFFUSION ASSAY

Staphylococcus aureus overnight grown cultures were in LB medium. The culture was centrifuged at 5000 rpm for 5 minutes. The obtained pellet was washed in 1 × PBS and again suspended in LB medium. Overnight culture (100 µl) was evenly distributed on LB plates and further incubated at (37 ° C) for 30 minutes. Next, a curcumin-loaded oocyte-xanthan gum film was exposed to a Staphylococcus aureus plate. Inhibition zones were observed after 24 and 48 hours of incubation.

IV RESULT AND DISCUSSION

Egg shell are considered to be one of the cheapest and the finest source of raw materials that has been extensively used in the field as bone substitutes i.e., hydroxyapatite and are also used in several other biomedical application. Here in the present work fine powdered form of such egg shells are used for the fabrication of the film as shown in Fig 1.

Fig 1: Fabrication of the biocompatible film from eggshell waste

Further the XRD analysis of the egg shell powder as shown in Fig 2 shows diffraction peaks that represent $CaCO_3$ illustrate the chemical composition of the egg.

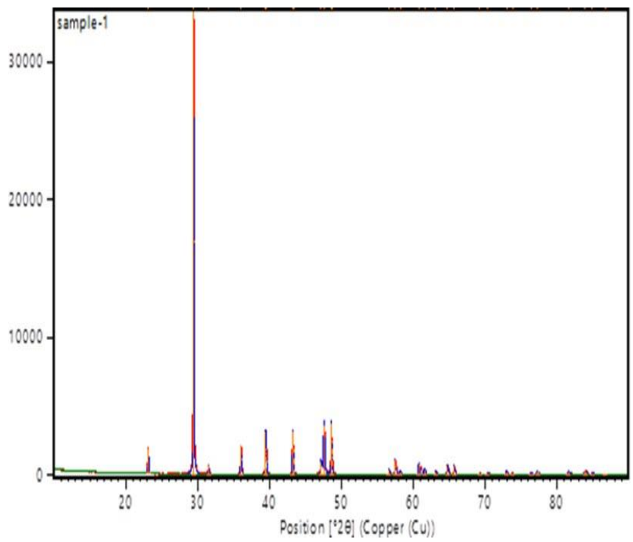

Fig 2: XRD analysis of Eggshell powder

XG is far superior to sodium hyaluronate because it is quite durable to enzymatic and hydrolysis reactions in vivo, making it the focus of biomedical applications. Intra-articular XG based injection of 0.5-2.0 wt% is a promising alternative treatment for osteoarthritis [16]. XG is an active ingredient in the food industry due to its non-toxicity, efficient thickening capacity, superior viscosity at low concentrations, and pseudoplastic behaviour is seen aqueous solution. In addition, it is highly stable over a wide temperature range and ionic strength [17-18]. Such behaviour of XA enhances the rate of miscibility, fluidity, and pumpability. However, there are major issues related with the application of natural XG. H. High swelling rate of hydration and high probability of microbial contamination. Therefore, acetic acid-modified XG chemically limits these major drawbacks suitable for drug delivery applications.

Fig 3: Zone of inhibition after 24h and 48h exposure

Curcumin has proven effective therapeutic uses, including anti-carcinogenic, antioxidant, anti-diabetic, antibacterial, and anti-inflammatory effects. However, the application of curcumin is severely hampered because it is primarily associated with challenges that impede therapeutic effects, such as low bioavailability, rapid systemic removal, reduced absorption, and high metabolism. Another drawback of curcumin is its low solubility. H. At about 11 ng / ml, at normal pH <7, the rate of decomposition of curcumin is very low i.e., >20% of the total curcumin decomposed in 1 hour. To overcome these major problems, potential dose levels should be administered at reduced doses. Therefore, curcumin nanoforms are manufactured to achieve better stability and controlled release of curcumin. Finally, to verify the effectiveness of the prepared eggshell patch, cotton swabs taken from the patient were collected and pathogen isolated, as shown in Figure 3. A curcumin-based patch was placed on an agar plate and showed some antipathogenic activity.

V *CONCLUSION*

So here we were able to create an eggshell-based XG patch with antibacterial effects in a simple process. Again, swelling of the fabricated XG based eggshell patch was observed and fluid intake was analysed. The modified XG matches in vitro non-uniform degradation and swelling rates. Overall, acetic acid modification has been shown to alter the bulk properties of XG, suggesting that it can be adjusted for used in orthopaedic applications. XRD analysis proves the presence of $CaCO_3$ in the eggshell. Eggshell and XG and their composites open many doors for creating new architectural and to be potentially used for biomedical application and wound healing applications such as bone tissue regeneration.

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REFERENCE

1. A.H. Parsons “Structure of the Eggshell egg shell quality ultra-structure membranes mammillary knob palisade layer surface crystal layer” vol 61, no. 10, pp 2013- 2021, Oct 1982.doi org/10.3382/ps.0612013.

2. A.M. King’ori, “A Review of the uses of poultry eggshells and shell membranes” International Journal of Poultry Science vol 10, no. 11. doi:10.3923/ijps.2011.908.912.

3. Mateus Carvalho Amaral, Fabrício Bagli Siqueira, André Zoteli Destefani, and José Nilson F. Holanda, “Soil–cement bricks

incorporated with eggshell waste" Proceedings of the Institution of Civil Engineers - Waste and Resource Management vol 166. doi.org/10.1680/warm.12.00024.

4. Margarida J. Quina, A.R Micaela, Soares Rosa Quinta-Ferreira "Applications of industrial eggshell as a valuable anthropogenic resource" vol 123, , pp 176-186, Aug 2017.doi.org/10.1016/j.resconrec.2016.09.027.

5. Maja Radziemska,Magdalena Daria Vaverková, Dana Adamcová, Martin Brtnický Zbigniew Mazur "Waste and Biomass Valorization" volume 10, pp 2537–2545 , doi.org/10.1007/s12649-018-0288-8.

6. Xinghua Mengand Da Deng, "Trash to Treasure: Waste Eggshells Used as Reactor and Template for Synthesis of Co9S8 Nanorod Arrays on Carbon Fibers for Energy Storage" Chem. Mater. 28, 3897–3904, doi.org/10.1021/acs.chemmater.6b01142.

7. Abideen I. Adeogun, Andrew E. Ofudje, Mopelola A. Idowu & Sarafadeen O. Kareem "Waste and Biomass Valorization" vol 9, pp 1469–1473 (2018) doi.org/10.1007/s12649-017-9891-3.

8. Nongnuch Laohavisuti, Banjong Boonchom, Wimonmat Boonmee, Kittichai Chaiseeda, and Somkiat Seesanong "Simple recycling of biowaste eggshells to various calcium phosphates for specific industries" 10: 2436. doi: 10.1038/s41598-020-59324-5.

9. Ferraz, E., Gamelas, J. A. F., Coroado, J., Monteiro, C. & Rocha, F. "Eggshell waste to produce building lime: calcium oxide reactivity, industrial, environmental and economic implications" vol- 115. doi.org/10.1617/s11527-018-1243-7 .

10. Muliwa, A. M., Leswifi, T. Y. & Onyango, M. S." Performance evaluation of eggshell waste material for remediation of acid mine drainage from coal dump leachate" Miner. Eng. Vol-122, 241–250, doi.org/10.1016/j.mineng.2018.04.009 (2018).

11. Cree, D. & Rutter, A. Sustainable bio-inspired limestone eggshell powder for potential industrialized applications. ACS Sustain. Chem. Eng. Vol 3, 941– 949, doi.org/10.1021/acssuschemeng.5b00035 .

12. Singh, V. K., Chevli, M. H., Tauseef, S. M. & Siddiqui, N. A." Treatment of effluent from pharmaceutical industry using calcium oxide obtained from eggshells". In: Siddiqui, N., Tauseef, S., Bansal, K. (eds.) Advances in Health and Environment Safety, pp. 327–343. Springer, Singapore (2018)," "Valorization of Eggshell Biowaste for Sustainable Environmental Remediation" doi: 10.1038/s41598-020-59324-5.

13. Sarder, M. R., Hafiz, N. A. & Alamgir, M. Study on the effective reuse of eggshells as a resource recovery from municipal solid waste. In: Ghosh, S. (ed.) Waste Management and Resource Efficiency, pp. 71–79. Springer, Singapore (2019), "Valorization of Eggshell Biowaste for Sustainable Environmental Remediation" 10: 2436. doi: 10.1038/s41598-020-59324.

14. Ummartyotin, S. & Manuspiya, H. A critical "review of eggshell waste: An effective source of hydroxyapatite as photocatalyst". J. Met. Mat. Miner. 28, 124–135, vol 28 2018;124–135. doi: 10.14456/jmmm.2018.17.

15. Michel MilasMarguerite RinaudoMagali Knipper and Jean Luc Schuppiser "Flow and viscoelastic properties of xanthan gum solutions" 1990, vol- 23, 9, 2506–2511 tion Date:April 1, 1990doi.org/10.1021/ma00211a018.

16. Sarder, M. R., Hafiz, N. A. & Alamgir, M. Study on the effective reuse of eggshells as a resource recovery from municipal solid waste. In: Ghosh, S. (ed.) Waste Management and Resource Efficiency, pp. 71–79. Springer, Singapore (2019).

17. Ummartyotin S, Manuspiya H. A critical review of eggshell waste: An effective source of hydroxyapatite as photocatalyst. J. Met. Mat. Miner. 2018; 28:124–135. doi: 10.14456/jmmm.2018.17.

18. Milas M, Rinaudo M, Knipper M, et al. Flow and viscoelastic properties of xanthan gum solutions. Macromolecules. 1990, vol 23, no 9, pp 2506–2511.