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Biodegradable PHB–Alginate Composite Matrix for Controlled Endolysin Release

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INTRODUCTION & AIM

Modern approaches in tissue engineering and regenerative medicine require biomaterials that can simultaneously provide mechanical support to damaged tissues and serve as platforms for the localized delivery of biologically active molecules. For wound dressings and "bio-patches," it is essential to combine barrier properties, sufficient porosity for gas and mass exchange, and controlled release of therapeutic agents directly at the injury site. Electrospun fibrous matrices (fig. 1) are particularly attractive in this context due to their high surface area, tunable porosity, and ability to mimic the architecture of the extracellular matrix.

In this work, a composite bio-patch was developed in which an electrospun poly(3-hydroxybutyrate) (PHB) matrix provides mechanical integrity and a porous scaffold, while a Ca^{2+}-crosslinked alginate layer functions as a carrier for model proteins (endolysins) and enables their sustained local delivery. To obtain a thin and more homogeneous alginate coating, diffusion-based Ca^{2+} crosslinking through a dialysis membrane was employed, improving the structural reproducibility of the composite.

The aim of this study was to design and characterize a PHB–alginate composite bio-patch and to evaluate its structural, mechanical, and protein encapsulation and release properties.

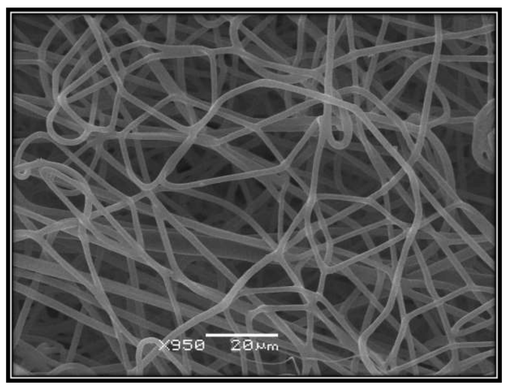

Figure 1. SEM image of the fibrous structure of the PHB matrix shows ultrafine fibers with diameters of 2–4 µm and random orientation, mimicking the extracellular matrix.

METHOD

The electrospun matrix was prepared from poly(3-hydroxybutyrate) (PHB) as the structural scaffold. A 1.5% (w/v) sodium alginate solution was used as the functional layer and ionically crosslinked with 5% $CaCl_2$. Model proteins, endolysins (0.5 µg/mL), were incorporated into the alginate to assess encapsulation and release. Gel formation was performed in dialysis bags, with a final gel volume of 1 mL, and protein content was determined by the Bradford assay at 595 nm. The composite was assembled by applying the alginate–protein solution in 20 mM HEPES buffer onto the PHB matrix, followed by Ca^{2+} crosslinking through a dialysis bag for approximately 20 min and subsequent washing. The composite was characterized by scanning electron microscopy, tensile testing, and Bradford analysis, and encapsulation and release efficiencies were calculated after 24 h washing in 22.5 mL HEPES buffer.

RESULTS & DISCUSSION

The results indicate that the alginate gel was successfully formed on the PHB matrix (fig. 2), producing a stable composite structure. The PHB scaffold served as a mechanically supportive porous base, while the Ca^{2+}-crosslinked alginate layer created a continuous functional coating on its surface. This confirms that the PHB matrix was suitable for supporting gel deposition and for maintaining the integrity of the composite bio-patch.

At the beginning of the experiment, the total protein amount in the system was 0.5 µg. After the loading, washing, and release steps, and following the calculations, the total recovered protein value was 0.401 µg. This indicates that a substantial fraction of the protein remained associated with the PHB–alginate composite, supporting effective encapsulation and retention within the gel layer. The graph (fig. 3) of the Bradford calibration curve confirmed the reliability of protein quantification and showed that the endolysin sample could be positioned on the standard line for concentration estimation.

In terms of release, the PHB-supported gel provided a sustained delivery behavior over 24 hours in HEPES buffer. This suggests that the gel was not simply washed away from the matrix, but remained attached enough to enable controlled release. Such behavior is advantageous for bio-patch applications, where the matrix should both stabilize the gel and help regulate release at the site of application.

Overall, the PHB matrix was not just a passive support but an essential structural component that enabled formation, stabilization, and functional performance of the alginate gel. The combination of initial protein loading and the recovered 0.401 µg total value demonstrates that the composite retained most of the incorporated protein while still allowing release, making the PHB–alginate system promising for localized delivery applications.

Figure 2. The finished composite bio-patch is a thin, semi-transparent film with a smooth surface, consisting of a PHB matrix coated with an alginate gel.

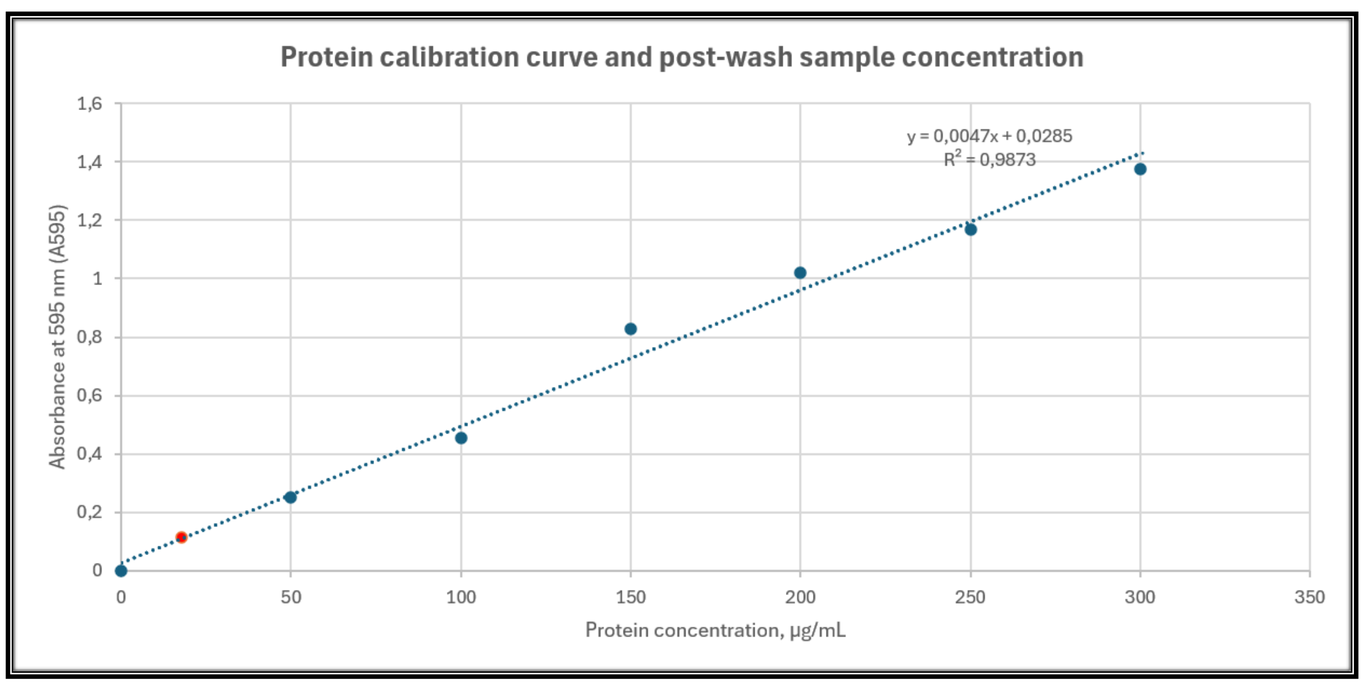

Figure 3. The blue dots and the blue calibration line represent the Bradford standard curve used for protein concentration. The red dot indicates the calculated result for endolysin, plotted on the trend line according to its measured absorbance (0,1124) which corresponds to 17,851 µg/ ml.

CONCLUSIONS

A biodegradable PHB–alginate composite bio-patch was successfully developed with a stable structure and good potential for localized delivery applications. The PHB matrix provided the mechanical support, while the Ca^{2+}-crosslinked alginate layer served as a functional carrier for endolysin.

The system showed effective protein retention and release behavior. From an initial protein amount of 0.5 µg, the calculated final recovered value after washing and release was 0.401 µg, indicating that most of the protein remained associated with the composite while a portion was released in a controlled manner. Overall, the results confirm that the developed material is a promising fully biodegradable platform for bioactive delivery.

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