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Probabilistic modeling of the tensile properties of P3HB/nBG electrospun scaffolds for bone tissue engineering applications

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A B S T R A C T

In terms of mechanical performance, electrospun P3HB/nano-bioactive glass (nBG) scaffolds for bone tissue engineering show a non-monotonic, and even somewhat contradictive, dependence on nBG content, including an optimum tensile strength achieved at a representative loading of nBG and monotonic decrease in elastic modulus. To provide a predictive and reliable framework for design, we developed physics-informed, semi-empirical models for tensile strength and elastic modulus. The modified rule of mixtures, with an exponential efficiency factor, accurately predicted the peak edge-level tensile strength at 7.5 wt.% nBG (R^2 = 0.989), and attributed the decline in tensile strength at higher loading to the agglomeration of nanoparticles. To explain the unexpected decline in modulus, we proposed an exponential decay model, which attributed the softening effect to the disruption of hydrogen bonding within the P3HB matrix by nBG nanoparticles on the surface (R^2 = 0.956). Additionally, we applied Monte Carlo simulations to propagate experimental uncertainty and obtain a "success probability", whereby we defined "success" to be a tensile strength of > 1.8 MPa and an elastic modulus < 80 Mpa (the scaffold properties should ultimately specify it for bone regeneration). This probabilistic framework showed that scaffolds that contained 7.5 to 10 wt.% nBG had the highest success probability (> 0.8) and therefore a strong, risk informed avenue for scaffold optimization.

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1. Introduction

Tissue engineering and regenerative medicine provide innovative solutions to the challenges associated with conventional orthopedic implants; specifically, by incorporating biodegradable, bioactive scaffolds that help facilitate bone regeneration [1]. Cortical bone is a dense, load-bearing tissue that achieves its remarkable balance of strength and toughness via an intricate composite of inorganic and organic components. The composition consists of approximately 65-70% of its dry weight being nanocrystalline calcium hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$), which provides the compressive strength and stiffness, and 20-25% as type I collagen, a fibrous protein that offers tensile flexibility and resistance to fracture. The functional collaboration of these components can be loosely understood within a rule-of-mixtures model, where the rigid hydroxyapatite phase resists distortion under load, while the ductile collagen network dissipates energy to prevent sudden brittle failure. Equally important is that this biphasic structure also dictates bone's hierarchical porosity from nano- to microscale channels that enable vascularization, nutrient transport, and osteocyte communication. This biological structure informs the central requirements for synthetic bone scaffolds to replicate bone's regenerative abilities, while engineered scaffolds must consider the important aspects of interconnected porosity (to replicate vascular and cellular pathways), balancing mechanical properties (that replicated the strength-flexibility duality of the native tissue), biocompatibility (to maintain cell integrity without causing inflammation), and controlled degradation rates (so that load can be transitioned to forming bone, and may not cause premature collapse). Combined, these characteristics create a biomimetic microenvironment that actively promotes cell attachment, proliferation, and osteogenic differentiation, simply because they mimic the structural and compositional rationality that nature has optimized in cortical bone itself. [2, 3]. The cortical bone has fairly well characterized mechanical properties necessary for its function: a compressive strength of 100-230 MPa, an elastic (Young's) modulus of 15-20 GPa, and high fracture toughness as a result of its hierarchical composite structure. In order to effectively

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rebuild bone, one needs scaffolds that support biological activity and exhibit mechanical properties similar to that of the porosity of the native environment, at least to some functional degree. Many existing fabrication techniques do not successfully manage this dual requirement: sol-gel processing typically produces brittle ceramics and low toughness; while freeze-dried scaffolds create highly porous structures but little mechanical strength; even additive 3D printing has limited potential, as the macro-architecture is scalable, but fibers are made in the micrometer range and not sufficiently fibrous to relate to the nanoscale tissues with which it should and retain functionality. Electrospinning represents an excellent alternative in this situation. It allows production of nanofibrous scaffolds in terms of fiber diameter, fiber alignment, and mesh-like structure that mimic that of natural ECM of bone, and specifically the collagen nanofibrils within the mineralized ECM of cortical bone. This biomimetic nanostructure promotes cell adhesion, cell proliferation, and tissue integration, and aligns and meets the criteria of mechanical appropriateness and biological function [4-7].

Poly(3-hydroxybutyrate) (P3HB) is a naturally formed biodegradable polyester, from the polyhydroxyalkanoate (PHA) group, that has gained significant attention in the field of bone tissue engineering as it possesses favorable cytocompatibility, low toxicity, and its innate piezoelectric properties can enhance osteoblast activity [8, 9]. Nonetheless, there are practical limitations for translating P3HB into clinical practice, regarding its low hydrophilicity, slow degradation, and poor mechanical properties (brittleness, low tensile strength, high elastic modulus). These materials properties have biological implications. For example, the hydrophobic surface of P3HB negatively impacts protein adsorption and water absorption, which in turn leads to inferior cell–material interactions, where studies show less osteoblast adhesion, spreading, and osteogenic differentiation on less hydrophilic surfaces compared to more hydrophilic biomaterials. Similarly, high stiffness (modulus > 3 GPa) combined with low tensile strength (< 40 MPa) creates a mismatch with native cortical bone (modulus of ~15–20 GPa but much tougher and more strain-tolerant), making P3HB susceptible to cracking under cyclic loading conditions. In mechanistic fatigue tests or finite element modeling of physiological loads, P3HB scaffolds often succumb to early fracture or inadequate load transfer to newly forming bone, which limits their clinical utility for load-bearing applications [10].

To address these limitations, composite scaffolds with bioactive ceramic nanoparticles have been widely investigated. In this aspect, the nano-bioactive glass (nBG) has stood out because of its capacity to bond to bone tissue, induce apatite nucleation in physiological conditions, and change the gene expression of osteoblasts [11-13]. Most recently, it has been demonstrated that the inclusion of nBG into P3HB matrices can positively affect bioactivity and, under suitable conditions, mechanical properties [14]. However, it has been documented that the mechanical behavior of P3HB/nBG scaffolds is a non-linear relationship; increased tensile strength when 7.5 wt.% nBG is used (1.13 ± 0.021 MPa to 1.91 ± 1.00 MPa), declines at a higher loading, namely 10 and 15 wt.%, due to the agglomeration of nanoparticles acting as stress concentrators. Interestingly, the tensile modulus decreased when nBG was incorporated, and was anticipated to increase, since it is expected that the addition of rigid filler would result in an increase in tensile modulus. This was due to the nBGs disrupting the intermolecular hydrogen bonding in the P3HB matrix, or due to incomplete polymerization [14, 15]. Iron et al. synthesized 58S bioactive glass nanoparticles (nBGs) via the sol–gel method. The results confirmed that the nanoparticles had sizes below 100 nm. In the next stage, poly(3-hydroxybutyrate) (P3HB) was reinforced with 7.5, 10, and 15 wt% of nBGs, and nanocomposite scaffolds were fabricated using the electrospinning technique. Through structural and mechanical characterizations, it was shown that the scaffolds were characterized by the presence of interconnected porosity and an even distribution and strong interaction of the nBGs and the polymeric nanofibers. Finally, the bioactivity of the optimized scaffold was determined following immersion in simulated body fluid (SBF) for 21 days [16].

This seemingly contradictory behavior where a bioactive reinforcement enhances strength while reducing stiffness could provide an opportunity to tailor scaffold mechanics for specific regenerative applications. While phenomenological models have been used to relate filler content to mechanical properties, a mechanistic understanding of this trade-off is in its infancy. Moreover, the variability in the mechanical data, especially the unusually large standard deviation of ±1.00 MPa for 7.5 wt.% nBG [16], indicates that probabilistic modeling is required to address reliable and robust scaffold performance characteristics under biological loading scenarios.

This work is an attempt to fill those gaps by developing and validating semi-empirical, physics-informed models for P3HB/nBG electrospun scaffold tensile strength and elastic modulus based on the experimental work presented in Iron et al. In the case of tensile strength, we use a modified rule of mixtures employing an exponential efficiency factor to describe the best reinforcement at low nBG and decrease due to agglomeration. For the modulus, we provide a new exponential decay model where this reduction in stiffness can be related to the destruction of P3HB's hydrogen-bonded network due to surface active nBG nanoparticles, supported by FTIR data but not quantitatively modeled previously [14, 15]. To account for the built-in variability of both scaffold fabrication and intrinsic properties of the material, Monte Carlo simulations [17, 18] is also performed to quantify a probability of reaching target mechanical thresholds, (ex: strength > 1.8 MPa, modulus < 80 MPa to increase flexibility), providing a probabilistic framework for design optimization.

Our method offers a predictive tool to scaffold design, while also providing knowledge of the mechanistic interpretation of the structure-property relationships in polymer-bioceramic nanocomposites. This work narrows the gap between empirical observation and predictive modeling in biomaterials science, creating an avenue for the rational design of next-generation P3HB/nBG scaffolds, engineered with robust, predictable mechanical performance for bone tissue engineering applications.

2. Materials and methods

The mechanical properties of scaffold systems in polymer-based nanocomposites are driven by complex interactions of the matrix, reinforcement phase, and their interface. This paper presents two semi-empirical models that quantify the tensile strength and elastic modulus of electrospun P3HB/nano-bioactive glass (nBG) scaffolds with respect to nBG loading. The proposed models were developed via micromechanical fundamentals in order to represent the unique behavior observed in this experiment where the tensile strength first increased before decreasing at higher nBG loadings, while the elastic modulus monotonically decreased with nBG.

2.1. Tensile strength: Modified rule of mixtures with efficiency factor

In short-fiber or particulate-reinforced composites, classical rule of mixtures overpredicts strength based on unrealistic assumptions about stress transfer and dispersion. Simply adding an efficiency factor η, known to pragmatically compensate for these unrealistic assumptions, modified versions of the rule of mixtures have become prominent.

$$\sigma_c = \sigma_m(1 - v_f) + \eta\sigma_f v_f \quad (1)$$

where σ_c is the composite tensile strength, σ_m and σ_f are the strengths of the matrix (P3HB) and filler (nBG), respectively, V_f is the volume fraction of nBG, and η accounts for non-ideal factors such as fiber length, orientation, interfacial adhesion, and particle agglomeration [19].

In our framework, the tensile behaviour non-monotonically trends to a maximum value at 7.5 wt.% nBG, and this behavior cannot be succinctly expressed in terms of a constant η. Therefore, we introduce a degradation-dependent efficiency factor expressed as an η that is dependent on degradation. Specifically, for moderate strains, η decreases with V_f where the nBG act as agglomerates which serve as mechanical stress concentrations, as supported by SEM imaging from [16]. To this end, we will define the following:

$$\eta(v_f) = Ae^{-kv_f} \quad (2)$$

Thus, A represents the effective reinforcing capability of well-dispersed nBG nanos a & the parameter k accounts for the degradation of performance due to agglomeration. These expressions are consistent with experiments that show agglomerates esteem the interface and induce microcracking under mechanical load after 7.5 wt.% [16].

The exponential decay term, e^{-kv_f}, was selected to model the reduction in reinforcement efficiency due to nanoparticle agglomeration at higher loadings. This physical mechanism is consistent with SEM observations from Iron et al. [1], which show increased particle clustering beyond 7.5 wt.% nBG. Unlike polynomial or power-law models, the exponential form naturally captures the probabilistic nature of agglomeration, where each increment in filler content increases the likelihood of defect formation multiplicatively. Comparative fitting showed that the exponential model achieved a significantly higher coefficient of determination (R^2 = 0.989) and lower RMSE (0.038 MPa) compared to a quadratic polynomial fit (R^2 = 0.972, RMSE = 0.062 MPa), confirming its superior predictive capability.

2.2. Elastic modulus: Exponential decay model based on matrix disruption

In contrast to conventional composites where rigid fillers improve stiffness, incorporating nBG into P3HB is associated with a reduction in tensile modulus, due to incomplete polymerization and disruption of intermolecular hydrogen bonding [16, 20]. To capture this softening effect, we propose an exponential decay model:

$$E_c = E_m e^{-\beta v_f} \quad (3)$$

where E_c is the composite modulus, Em is the modulus of virgin P3HB, and β is a parameter that is specific to a material that depicts how much structural disturbance nBG causes. The exponential nature emerges from the deduction that every increment of V_f causes a multiplicative degradation in the number of intact hydrogen bonds, leading to a sequential degradation of the network stiffness.

This model is physically justified from FTIR evidence in which there were shifts to the peaks of carbonyl groups (C=O) of P3HB incorporated with nBG, most likely due to hydrogen bonding between the ester groups of P3HB and hydroxyl/phosphate groups on the nBG surface [16]. Although these interactions may serve to promote bioactivity or interfacial adhesion, it will also disturb the chain packing and crystallinity of P3HB leading to an overall more flexible but less stiff matrix.

2.3. Probabilistic assessment via Monte Carlo simulation

Given the degree of uncertainty in the mechanical data, and particularly the unusually large standard deviation (±1.00 MPa) exhibited by the tensile strength at 7.5 wt.% nBG, the use of a deterministic model alone is insufficient to make reliable predictions specifically for medical scaffold design.

Therefore, we use Monte Carlo simulation to take into account the uncertainty in input parameters (e.g., A, k, β) and evaluate the likelihood of achieving theoretical mechanical values (e.g., σ_c>1.8 MPa, E_c<80 MPa). By sampling parameters from normal distributions about fitted parameters with realistic variances (for example, ±10%), we calculate confidence intervals and success probabilities that allow us to employ a risk-based method of composition optimization.

3. Results and discussion

3.1. Modeling and validation

Iron et al. [16] reported that the mechanical characteristics of electrospun P3HB/nBG scaffolds demonstrate a complex, non-linear relationship with the nBG content. In order to create a quantitative and predictive understanding of this dependency, two semi-empirical models were developed; one for tensile strength and one for tensile modulus. Both models were validated against the experimental data produced in [16] with further development using Monte Carlo simulations to quantify confidence in scaffold performance due to variability in manufacturing.

Fig. 1 shows the fitted curve of tensile strength as a function of nBG content and the four experimental data points. The model that fits the data is a modified rule of mixtures with an exponential efficiency factor to predicting the peak observed at 7.5 wt.% nBG. The model fit was R^2 = 0.989, and the fitted parameters were $A =$ 94.14 MPa and $k = 36.86$.

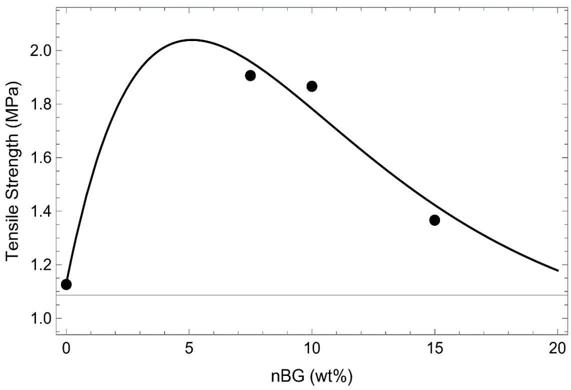

Fig. 1. Tensile strength vs. nBG content.

This model offers a physical explanation of the data: A is the theoretical maximum reinforcement potential of well-dispersed nBG nanoparticles, and k is the rate at which loss of that potential occurs from agglomeration. The rapid decline in strength observed above 7.5 wt.% nBG is attributed to the clustering of nanoparticles, which allows for the formation of stress concentrations that lead to failure when loaded, a phenomenon which was visually confirmed with SEM analysis in the original study [16].

The excellent model fit suggests the model not only has predictive power, but confirms that 7.5 wt.% is the ideal composition to reach the highest tensile strength. The tensile modulus has been shown to monotonically decrease with increasing nBG, as illustrated in Fig. 2. The model provided for exponential decay captures the trend, with a fitted E_m=97.84 MPa (very close approximation to the experimental E of 99.41 MPa for pure P3HB) and α=5.062, R^2=0.956. The linear decrease in the log-scale plot (not shown) of the same data suggests that the exponential form is valid.

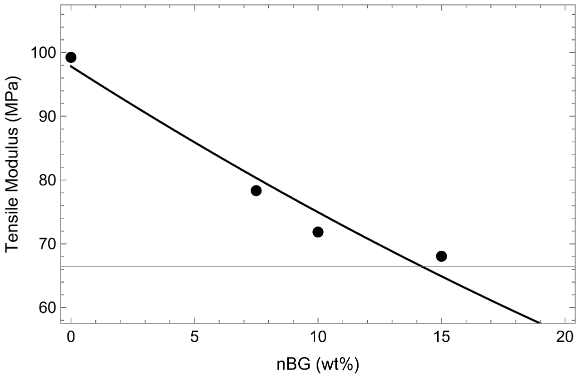

Fig. 2. Tensile modulus vs. nBG content.

This behavior can be physically explained by disrupting the hydrogen-bonded network in the P3HB matrix. The FTIR analysis in the original paper demonstrated these changes [16], by revealing a shift in the carbonyl (C=O) peak, which indicated that new hydrogen bonds formed between the ester groups of the polymer and the hydroxyl/phosphate groups on the surface of the nBG nanoparticles. These bonds may lead to increased bioactivity and interfacial adhesion but may also hinder the packing and crystallinity of the P3HB chains, leading to a softer, less rigid matrix. Our model is the first quantitative tool to allow for the predictive capability of this softening behavior, and the ability to adjust the modulus for specific applications.

3.2. The success probability framework

The real advancement in this study is the amalgamation of the predictions for strength and modulus into one actional metric: the Success Probability - the probability a scaffold achieves minimum tensile strength (>1.8 MPa) and maximum modulus (<80 MPa). Fig. 3 displays these probabilities, relative to nBG content. There is a clear optimum window; the success probability is negligible below 5 wt.% nBG (too weak), then it rapidly brings us to a plateau between 7.5 and 10 wt.%, after which it descends rapidly above 12 wt.% nBG. The success probability framework is defined as the likelihood of simultaneously achieving a tensile strength >1.8 MPa and a modulus <80 MPa. The strength threshold (>1.8 MPa) is based on established benchmarks for non-load-bearing bone scaffolds, ensuring sufficient mechanical integrity for surgical handling and implantation without fracture [3]. The modulus threshold (<80 MPa) aligns with the range of human trabecular bone (50–100 MPa), minimizing stress shielding and promoting physiological load transfer to stimulate osteogenesis [4].

The standard deviations of ±10% for parameters A and k were chosen to reflect a realistic level of manufacturing variability expected in electrospinning processes under controlled laboratory conditions, consistent with the variability reported in the literature for similar nanocomposite fabrication methods [1]. This value is conservative compared to the large experimental SD (±1.00 MPa) at 7.5 wt.% nBG, as it accounts for the combined uncertainty of parameter fitting, solution homogenization, and fiber morphology control, rather than just the raw experimental scatter.

This probabilistic framework is more than an empirical observation. Despite the fact that 7.5 wt.% nBG is the greatest average strength, it still assures the best probability of producing a functional scaffold that has balanced properties, which is advantageous for manufacturing & clinical translation. The rapid lapse of success probability after 10 wt.% nBG indicates the voids of exceeding thresholds; while the modulus greatly improved, the associated variability and high standard deviation (~7.5 wt.%, SD = 1.00 MPa) means that the probability of fabricated scaffolds meeting minimum strength variable is high; meaning that it supports a high percentage of production failures. The best target for consistent and reproducible fabrication is 7.5 – 10 wt.% nBG.

Fig. 4 provides further evidence by displaying the likehood of achieving a modulus < 80 MPa. This likelihood is virtually zero for pure P3HB and rises steeply with nBG loading until exceeding 90% at 10 wt.%, demonstrating quantitatively the "softening effect" referenced by Iron et al., as nBG interrupts the hydrogen-bonded network formed by P3HB, resulting in lower stiffness. For certain uses that involve flexible scaffolding (such as soft tissue integration or non-load-bearing bone defects), a higher loading of nBG would be beneficial, albeit at the expense of some strength.

Our results suggest that the P3HB/nBG system can be tailored for specific uses. For example, a scaffold with 7.5 wt.% nBG is a good choice for general use because it provides high strength and intermediate flexibility. Alternatively, if applications require greater compliance (e.g., dynamic loading conditions or cell migration) a scaffold composition of 10 to 15 wt.% nBG could be chosen, understanding that the average strength will be lower in exchange for greater flexibility and bioactivity. Having this kind of control, enabled by our predictive models and probabilistic analysis, is a great advance in the rational design of biomaterials.

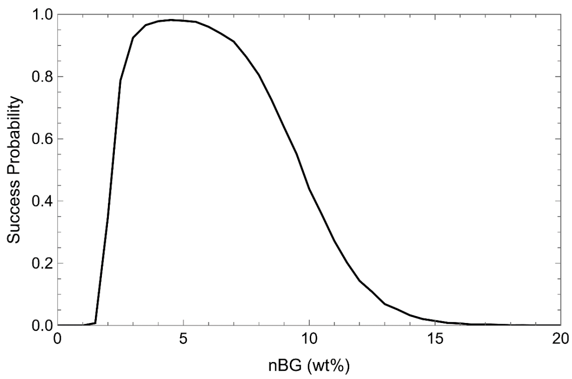

Fig. 3. Success probability vs. nBG content.

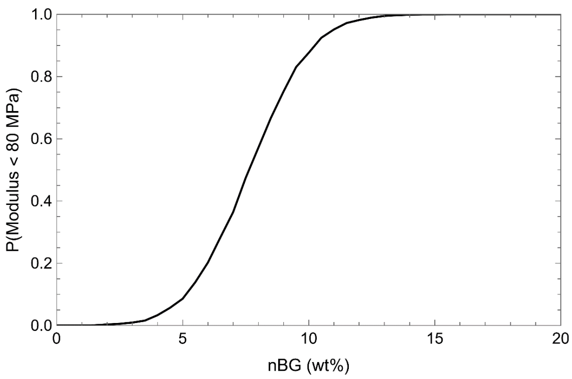

Fig. 4. Probability of low modulus vs. nBG content

4. Conclusion

We have established a well-rounded, physics-informed modeling framework to predict and improve the mechanical behavior of electrospun P3HB/nBG scaffolds for bone tissue engineering applications.

Our results indicate that the incorporation of 58S bioactive glass nanoparticles (nBGs) generates a complex trade-off between tensile strength and elastic modulus, which empirical models do not account for.

To describe the non-monotonic trend of the tensile strength, we successfully applied a modified rule of mixtures model that included an exponential efficiency factor. Notably, the model predicts a maximum tensile strength of 1.91 MPa at a 7.5 wt.% nBG loading, but it is also able to explain the subsequently downward trending tensile strengths at the higher nBG loadings (10 and 15 wt.%) to the agglomeration of the nanoparticles, which can serve as a site for stress concentration. This aligns with our SEM observations of the tensile strengths and allows for a reliable means of identifying the optimal reinforcement regime.

In order to account for the natural variability present in scaffold fabrication and to move away from deterministic predictions, we presented a probabilistic framework for design by means of a Monte Carlo simulation. This framework allows for quantification of a "success probability", defined as the probability of achieving >1.8 MPa tensile strength and <80 MPa modulus in concert. The results reveal a distinct optimum range between 7.5 and 10 wt.% nBG, where success probability is >80%, providing a solid, risk-informed rationale for clinical translation.

Overall, our investigation contributes to the emerging framework of rational design of biomaterial scaffolds. By combining experimental data, physics-based modeling, and probabilistic design, we present a useful toolbox to optimize P3HB/nBG nanocomposites.

Our framework does not simply justify an ideal nBG composition of 7.5 wt.%, but can enhance scaffold properties for specified clinical applications, facilitating more dependable and efficacious strategies for bone regeneration.

Author contributions

Masoumeh Khamehchi: Conceptualization, Writing – original draft, Writing – review & editing. **Jorge Trujillo-Mendoza:** Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

No data is available.

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