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Bio mimetic Enamel Regeneration: Current Strategies and Emerging Role of Elastin-Like Recombinamer Scaffolds

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Abstract

Background: Conventional restorative materials can restore dental function, but they do not entirely replicate the hierarchical architecture and biological properties of natural enamel. As a result, bio mimetic protein-based techniques have become increasingly popular as prospective strategies for encouraging enamel regeneration and remineralization.

Objective: This study assesses the present evidence for recombinant Elastin-Like Recombinamer-based bio mimetic scaffolds, namely those functionalized with statherin-derived mineral-binding peptides like SNA15, for guided enamel regeneration.

Methods: The relevant literature on ELR-based biomaterials, protein-guided enamel mineralization, statherin-derived peptides, recombinant protein synthesis, and bio mimetic remineralization were reviewed. Evidence from physicochemical, structural, mechanical, preclinical, and upcoming clinical research was evaluated, including characterisation methods such as FE-SEM, EDX, XRD, FTIR, Raman spectroscopy, and nano-indentation.

Current evidence and Key findings: Current research suggests that Elastin-Like Recombinamer-based scaffolds functionalized with mineral-binding peptides can promote organized hydroxyapatite nucleation and epitaxial crystal growth, resulting in enamel-like minerals with favorable structural, compositional, and mechanical properties.

Recombinant protein production and chromatography-free purification techniques, such as inverse temperature cycling, may enable scalable and cost-effective manufacturing. Preclinical and early translational investigations point to possible applications in enamel erosion, early carious lesions, and dentinal hypersensitivity.

Conclusion: Recombinant Elastin-Like Recombinamers-based bio mimetic scaffolds are a promising new platform for minimally invasive enamel regeneration. Their capacity to combine protein-guided mineralization with tailored scaffold functioning may overcome various limitations of traditional restorative techniques. However, the present information is primarily preclinical, and bigger, standardized clinical trials with long-term follow-up are needed to prove their safety, durability, and clinical relevance.

Keywords: Bio Mimetic Enamel Regeneration, Elastin-Like Recombinamers, Statherin-Derived Peptide, Protein-Guided Mineralization, Hydroxyapatite Remineralization, Regenerative Dentistry.

Introduction

Dental enamel is the toughest and most mineralized tissue in the human body, made up of roughly 96% carbonated hydroxyapatite crystals, 3% water, and less than 1% organic matrix. Its extraordinary hardness and wear resistance stem from the hierarchical structuring of elongated apatite Nano crystals into enamel rods and inter rod enamel, rather than just its high mineral content[1-3]. Unlike bone and dentin, mature enamel is entirely a cellular and a vascular, with no odontoblasts or resident progenitor cells capable of beginning tissue repair following eruption. As a result, enamel loss induced by tooth caries, erosive wear, abrasion, or trauma is deemed irreversible, leaving only prevention and restoration as clinical options[2-5].

Dental caries remain one of the most common chronic disorders worldwide. According to the World Health Organization (WHO), untreated dental caries impact almost 2.5 billion people worldwide, while erosive tooth wear is increasing rapidly due to food acids, Gastroesophageal reflux illness, and lifestyle changes [6,7]. Progressive enamel loss exposes the underlying dentin, resulting in hypersensitivity, increased susceptibility to bacterial invasion, impaired esthetics, and eventual tooth breakage if not addressed[4,6].

Restorative dentistry today mostly uses synthetic materials such as resin-based composites, glass ionomer cements, ceramics, and dental amalgams. Although these materials can restore tooth form and function, they cannot replicate the intricate anisotropic microstructure or biological interface of native enamel [8-10].

Polymerization shrinkage, thermal expansion mismatch, adhesive degradation, bacterial micro leakage, and recurrent caries remain key causes of restoration failure, frequently necessitating periodic replacement throughout a patient's lifetime[9-12]. Long-term clinical trials estimate annual restoration failure rates of 1% to 5%, depending on cavity size, material selection, and patient-related risk factors[10-12].

These constraints have prompted the development of regenerative and bio mimetic systems for rebuilding enamel via biological mineralization rather than artificial replacement. Bio mimetic mineralization aims to mimic the natural process of Amelogenesis by controlling the

nucleation, orientation, and hierarchical development of hydroxyapatite crystals under physiological settings[13-15]. During tooth development, enamel formation is controlled by extracellular matrix proteins including amelogenin, enamelin, ameloblastin, tuftelin and proteases such as matrix metalloproteinase-20 (MMP20) and kallikrein-4 (KLK4), which govern matrix breakdown and crystal elongation[14-16]. Replicating these biological cues with synthetic or recombinant protein scaffolds has thus become a primary goal in enamel tissue engineering.

Among the different bio mimetic techniques examined, intrinsically disordered proteins (IDPs) have received substantial attention because of their dynamic molecular conformations and capacity to regulate mineral nucleation thru flexible binding domains[17,18]. Many enamel matrix proteins, particularly amelogenin, have intrinsically disordered properties that allow for brief interactions with calcium and phosphate ions while directing highly organized crystal formation[17,19].

Mimicking these molecular features allows us to create synthetic matrices that can reproduce the spatial organization of natural enamel development.

Elastin-Like Recombinamers (ELRs) are a type of recombinant protein polymer that has been genetically modified to resemble tropoelastin. These biopolymers consist of repeating pent apeptide sequences, commonly Val-Pro-Gly-X-Gly (VPGXG), with the guest residue (X) changed to customize mechanical, biochemical, and self-assembly properties[20, 21]. ELRs have a reversible inverse temperature transition, which allows them to be purified using Inverse Transition Cycling (ITC) rather than conventional chromatography.

This technology significantly reduces manufacturing costs and produces highly pure recombinant proteins suited for biomedical applications[20-22].

Beyond their purifying benefits, ELRs have exceptional biocompatibility, biodegradability, and modularity. Bioactive peptide motifs can be genetically bonded to the ELR backbone to confer cell adhesion, enzymatic responsiveness, antibacterial activity, or mineral-binding capabilities while maintaining structural integrity[20,21]. ELRs' versatility has enabled them to act as inject able hydro gels, drug delivery vehicles, vascular scaffolds, cartilage matrices, and mineralization templates in regenerative medicine [20, 21, 23].

Among mineral-binding peptides, the statherin-derived N-terminal peptide (SNA15) is one of the most effective nucleation motifs for enamel bio mineralization. Native statherin is a salivary phosphoprotein that maintains calcium phosphate super saturation in saliva while also controlling hydroxyapatite crystal formation on enamel surfaces[24-26].

The synthetic peptide SNA15 retains the highly acidic N-terminal domain responsible for strong hydroxyapatite affinity and has demonstrated the ability to promote organized calcium phosphate nucleation, stabilize amorphous calcium phosphate precursors, and facilitate epitaxial crystal growth aligned with native enamel crystallography [25-28].

Despite promising laboratory investigations, various obstacles remain to impede clinical translation of bio mimetic enamel regeneration. Large-scale recombinant protein manufacturing necessitates powerful microbial expression methods capable of producing high protein yields while retaining structural integrity.

Furthermore, remaining bacterial endo toxins must be effectively eliminated to meet clinical safety standards. Additional hurdles include obtaining consistent crystal orientation under physiological settings, maintaining long-term adhesion to partially demineralized enamel,

and demonstrating therapeutic efficacy in human subjects[21,22, 29].

Recent advancements in recombinant protein engineering, synthetic biology, and nanostructured nano materials have helped to break down these translational obstacles. Improved bacterial expression platforms, better fermentation methodologies, and chromatography - free purification approaches have enabled scalable manufacture of clinical-grade ELRs while reducing manufacturing complexity[20-22].

High-resolution characterization techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, and energy-dispersive X-ray spectroscopy (EDS) allow for comprehensive evaluation of crystal orientation, chemical composition, and mineral maturity during bio mimetic mineralization[13,30-32].

Table 1: Comparison of Enamel Regeneration Strategies.

Approach	Mechanism	Advantages	Limitations	Clinical Status
Fluoride	Remineralization	Simple	Superficial only	Clinical
CPP-ACP	Calcium delivery	Non-invasive	Weak structure	Clinical
Peptides	Bio mimetic	Good structure	Expensive	Experimental
ELR	Scaffold-guided	Highly promising	Early stage	Preclinical

Recent discoveries in regenerative dentistry have demonstrated that recombinant ELR-based biomaterials functionalized with the SNA15 mineral-binding peptide are attractive platforms for biological enamel regeneration. Current research focuses on the development of recombinant ELR scaffolds, scalable microbial production systems, and effective purification technologies, such as chromatography-free inverse temperature cycling, to provide high-purity biomaterials suited for translational applications. Preclinical investigations have shown that these bio mimetic matrices can induce guided epitaxial hydroxyapatite development on demineralized enamel surfaces while maintaining structural and compositional features similar to native enamel. Emerging translational and clinical studies point to possible improvements in the therapy of enamel erosion and dentinal hypersensitivity with less invasive chair side applications. Collectively, our findings show the potential of ELR-SNA15 bio mimetic scaffolds as a therapeutically applicable method for biological enamel regeneration, as well as the necessity for bigger clinical trials to confirm their long-term safety, efficacy, and durability in restorative dentistry.

Methods

Genetic Design and Recombinant Expression of ELR-SNA15

A synthetic gene expressing an ELR made up of repeating pent apeptide motifs based on the conventional VPGXG sequence was developed for recombinant production in Escherichia coli. The guest residue (X) was selectively changed with negatively charged amino acids to improve mineral-binding interactions and control the ELR's thermos-responsive transition behaviour. Codon optimization was implemented to improve recombinant protein expression in the chosen host system [20, 21, 23]. The ELR backbone was genetically fused at its C-terminus with the statherin-derived mineral-binding peptide SNA15 (DDDEEK), which has previously exhibited significant affinity for hydroxyapatite surfaces and the capacity to produce enamel-like crystal nucleation [25-27].

The recombinant construct was commercially manufactured and cloned into the pET-28a (+) expression vector using the bacteriophage T7 promoter.

An N-terminal His-tag was added to aid in the identification and purification of recombinant proteins during process development. The resultant plasmid was then injected into chemically competent Escherichia coli BL21 (DE3) cells using a typical heat-shock transformation procedure[33,34]. The presence of the recombinant insert. Clones that produced the expected amplicon size were then subjected to Sanger sequencing to verify the identity and sequence integrity of the inserted ELR-SNA15 construct prior to recombinant protein production studies [35, 36].

Recombinant ELR synthesis in E. coli is typically started with antibiotic-selected starter cultures cultured in LB medium, followed by transferring an appropriate seed volume into stirred-tank bioreactors using Terrific Broth. During fermentation, essential process parameters such as dissolved oxygen, agitation, aeration, and pH are closely monitored to ensure high - cell density development and consistent recombinant protein expression.

Typical bioreactor settings include maintaining dissolved oxygen above roughly 30% saturation by automated agitation and aeration, while pH is controlled near neutrality using proper acid-base addition techniques [37]. Controlled fermentation methods enable scalable and repeatable production of recombinant ELR biomaterials [38-42].

Recombinant ELR production in E. coli is often stimulated during the mid - exponential growth phase by IPTG. To increase soluble protein expression while minimizing inclusion-body formation, the cultivation temperature is typically reduced. Following the expression phase, bacterial biomass is normally harvested via chilled centrifugation and stored under freezing conditions prior to downstream purification [34,43].

Purification of Recombinant ELRs by ITC

Following recombinant expression, bacterial cells are typically resuspended in a buffered solution containing protease inhibitors and mechanically destroyed using probe sonication. Sonication is often carried out under regulated, intermittent settings with cooling to limit heat generation and safeguard the structural integrity of the recombinant ELR proteins[37].

After cell rupture, bacterial lysates are routinely cleared to remove insoluble cellular debris before being processed further. Purification of Elastin - like Recombinamer can utilize their characteristic lower critical solution temperature (LCST) behaviour thru ITC, a chromatography-free purification approach that allows for selective recovery of ELRs based on their reversible temperature-dependent phase change[22].

ELR purification by inverse temperature cycling is based on these polymers' reversible temperature-dependent phase shift. Controlled heating increases ELR aggregation and separation from soluble impurities, whereas subsequent chilling allows the purified protein to be resolubilized. Repeated hot-cold cycling can increase protein purity by utilizing variations in the phase transition behaviour of ELRs and host-cell contaminants [21,22,43].

Triton X-114 phase separation, a well-known method for eliminating endo toxins from recombinant protein preparations, can help to reduce residual lipo polysaccharide (LPS) contamination. Triton X-114 promotes the partitioning of hydrophobic endo toxins into the detergent-rich phase, allowing for the recovery of the recombinant protein in the aqueous phase. Repeated phase-separation cycles can enhance endo toxin elimination and protein purity[38, 44-46]. Following endo

toxin removal, the purified ELR preparation can be subjected to extensive dialysis against endo toxin - free water using an appropriate molecular-weight cut-off membrane, followed by sterile filtration, lyophilisation, and low-temperature storage to maintain protein stability prior to subsequent characterization or bio mimetic mineralization studies[45, 47].

Protein Characterization& Quality Assessment

The characterization of recombinant ELR-based biomaterials often includes complimentary biochemical and physicochemical analyzes. Protein content can be evaluated using colorimetric tests such as the bicinchoninic acid (BCA) method, which commonly uses bovine serum albumin as a reference standard[48]. Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) with Coomassie staining is commonly used to assess protein purity and apparent molecular size, while MALDI-TOF mass spectrometry can provide additional confirmation of molecular identity[49].

Endo toxin assessment is a critical quality control step for biomaterials intended for biomedical or clinical applications. This is frequently performed using Limulus Amebocyte Lysate (LAL)-based tests in conformity with appropriate pharmacopeial standards[50]. Furthermore, the inverse transition temperature (Tt) of ELRs can be characterized by monitoring temperature-dependent changes in optical density using ultraviolet-visible spectroscopy, providing information on their thermos-responsive behaviour and suitability for processing and biomaterial applications[38].

Bio mimetic Enamel Remineralization

In vitro research frequently use newly extracted human teeth, particularly premolars obtained during orthodontic extraction, to create therapeutically relevant enamel regeneration models. Teeth with fissures, restorations, fluorosis, or developmental problems are often excluded, followed by standardized cleaning and storage before research [51]. Controlled enamel demineralization is often accomplished by constructing defined enamel windows and using phosphoric acid-based etching methods to disclose the underlying mineralized architecture [52].

Following demineralization, protein-based bio mimetic scaffolds, such as ELR formulations, can be placed to the exposed enamel surface to aid in adsorption and serve as a template for subsequent mineral nucleation. The treated specimens are then routinely incubated in supersaturated artificial saliva or bio mimetic mineralization solutions including calcium and phosphate ions, as well as chosen fluoride and buffering components, at physiological temperatures and pH levels. These systems provide a controlled environment to evaluate protein-mediated hydroxyapatite nucleation, crystal development, and integration with the native enamel substrate[13,15].

This model allows for an assessment of ELR-based scaffolds' ability to recreate elements of natural enamel mineralization while also providing consistent conditions for further morphological, compositional, crystallo graphic, and mechanical characterization.

Surface Morphology and Mineral Analysis

The structural organization of bio mimetic enamel is usually evaluated using field-emission scanning electron microscopy (FE-SEM), which examines surface appearance, crystal development, and the structure of freshly generated mineral phases. EDS is typically combined with electron microscopy to identify elemental composition and calculate the calcium-to-phosphorus (Ca/P) ratio of regenerated minerals. Complementary techniques like XRD and FTIR provide information on the crystalline phase and chemical composition, whereas Raman spectroscopy can evaluate mineral maturation, phosphate bonding, and carbonate inclusion. These analytical methodologies give complementary data for

analyzing the structural and physicochemical similarity between regenerated enamel and native enamel[19,30-32].

Nano-Mechanical Characterization

Instrumented nano-indentation is routinely used to examine the mechanical performance of bio mimetic enamel, allowing for quantitative measurements of nano-hardness and elastic modulus.

Typically, a Berkovich diamond indenter is used in studies, with mechanical parameters determined using the Oliver-Pharr method based on load displacement data. Multiple indentations across each specimen are often undertaken to account for local variations in enamel structure and provide representative assessments of mechanical recovery[53].

Literature Synthesis

Expression Yields and Endo toxin Thresholds

The bench top bioreactor runs could produce a constant amount of pure ELR-SNA15 protein. SDS-PAGE examination should provide a single conspicuous band at the target molecular weight, with no visible degradation fragments[47,54.] Triton X-114 phase separation has been demonstrated as an effective strategy for reducing lipopolysaccharide contamination from recombinant protein preparations. Recent work indicates that combining Triton X-114 with complementary washing approaches can achieve substantial endo toxin reduction while preserving protein quality[35, 49, 55].

Instead of assuming a present threshold, final endo toxin concentrations should be quantitatively evaluated using a validated endo toxin assay and proven to fall within the applicable regulatory limit for the intended clinical application [48, 55].

Epitaxial Mineralization Kinetics and Mineral Composition

FE-SEM is commonly used to assess the morphology and organization of bio mimetically regenerated enamel after treatment with protein-based scaffolds. Studies on recombinant ELR-based matrices functionalized with mineral - binding peptides consistently show the production of continuous, compact, and well-integrated enamel-like mineral layers on demineralized enamel surfaces. These regenerated layers make intimate contact with the underlying enamel substrate, with little evidence of interfacial gaps or delamination, indicating excellent bio mimetic integration[15, 56, 57].

Pre-clinical morphological analyzes revealed that the regenerated mineral phase is primarily composed of elongated, needle-like hydroxyapatite crystals organized in highly ordered parallel bundles perpendicular to the enamel surface. This organization closely resembles the hierarchical prism architecture of native enamel and contrasts sharply with the irregular, randomly oriented calcium phosphate deposits typically observed in untreated or conventionally remineralized specimens, where crystal growth is frequently discontinuous and poorly organized [58-60].

Time-course studies indicate that bio mimetic enamel regeneration occurs in a manner similar to natural bio mineralization. Early mineral deposition is distinguished by the development of amorphous calcium phosphate (ACP) nanoparticles, which eventually change into elongated hydroxyapatite crystallites via guided crystal growth. As mineralization advances, the regenerated layer becomes progressively thick and strongly orientated, indicating the ability of ELR-based scaffolds and statherin-derived peptides to govern nucleation, crystal maturation, and epitaxial alignment via unique mineral-binding interactions[31, 57,61].

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$$\text{Ca/P Ratio} = \frac{\text{Atomic \% Calcium}}{\text{Atomic \% Phosphorus}} = 1.65 \pm 0.03$$

Complementary physicochemical analysis with XRD, FTIR, and Raman spectroscopy can demonstrate the effective production of crystalline hydroxyapatite. XRD analyzes revealed distinct hydroxyapatite diffraction peaks with preferential c-axis orientation, which closely resembled the crystalline organization of native enamel. FTIR can detect phosphate and carbonate vibrational bands in biological apatite, while Raman spectroscopy can identify the phosphate v1 symmetric stretching band near 960 cm^{-1}, indicating mineral maturation and increased crystallinity[57,59,60].

These previous study findings may suggest that recombinant ELR-based bio mimetic scaffolds, particularly those functionalized with statherin-derived mineral - binding peptides, effectively promote guided epitaxial hydroxyapatite formation and replicate many of the structural, compositional, and crystallographic properties of native human enamel.

These previous findings support the potential of next - generation biomaterials for minimally invasive enamel regeneration and highlight their promise for future clinical translation in restorative dentistry; further long-term clinical studies are required to validate their therapeutic effectiveness.

Mechanical Restoration Indices

Studies using instrumented nano-indentation could indicate that bio mimetic remineralization techniques can significantly enhance the mechanical characteristics of demineralized enamel, resulting in higher surface nano-hardness and elastic modulus compared to untreated lesions[15, 56, 59]. Protein-guided mineralization techniques have been shown to improve the mechanical properties of demineralized enamel to those of healthy native enamel. This enhancement is connected with better mineral deposition and organization, resulting in increased hardness and resistance to mechanical deformation[15, 56, 57]. Nano-indentation load-displacement analyzes reported in bio mimetic remineralization studies demonstrate reduced penetration depth and enhanced elastic recovery following treatment, indicating the formation of mechanically stable mineral layers with improved structural integrity and resistance to deformation[15, 59].

Several studies have also reported excellent interfacial integration between newly formed hydroxyapatite crystals and the underlying enamel substrate, with minimal evidence of interfacial debonding or delamination during mechanical evaluation. This highlights the ability of protein-based scaffolds to promote durable enamel repair [56,60.]Preclinical studies show that bio mimetic scaffolds based on recombinant ELRs, Amelogenin-inspired proteins, and statherin - derived peptides can restore not only the hierarchical organization of enamel, but also key functional mechanical properties required for long-term clinical performance [31,56, 61]. Despite these promising results, the current evidence is primarily based on in vitro and preclinical research. More well-designed clinical studies with standardized protocols and long-term follow-up are required to establish the durability, safety, and clinical effectiveness of ELR-mediated enamel regeneration and

its potential inclusion into routine restorative dentistry [57,62].

Overall, ELR-guided bio mimetic mineralization has promise for restoring the structural and mechanical features of demineralized enamel. However, larger clinical trials with long-term follow-up are required to determine its safety, durability, and therapeutic efficacy.

Discussion

Overall, the literature indicates that recombinant ELR-SNA15 scaffolds can promote regulated epitaxial mineralization and the production of enamel-like structures with advantageous structural, compositional, and mechanical properties. ELR-based devices provide a bio mimetic framework for directed crystal nucleation and growth, making them a promising minimally invasive method to enamel regeneration. Additional clinical validation is needed to determine their long-term translational potential[20, 21, 23, 25-27].

One of the most difficult aspects of enamel regeneration is adult enamel's incapacity to mend itself. During tooth development, ameloblasts secrete a highly organized extracellular matrix containing amelogenin, enamelin, and ameloblastin, which together control crystal nucleation, orientation, elongation, and maturation. After tooth eruption, these cells disappear, and the organic matrix is totally destroyed, leaving adult enamel without any regenerating capacity[14-16]. As a result, successful bio mimetic regeneration necessitates the use of an artificial matrix capable of replicating at least part of the structural and metabolic functions previously provided by enamel matrix proteins.

Recombinant ELR-SNA15 constructs have emerged as promising bio mimetic platforms that mimic key functional properties of amelogenin while improving stability, repeatability, and manufacturing scalability. ELRs have intrinsically disordered and highly flexible molecular conformations that allow for spontaneous self-assembly into nanoscale fibrillar networks under normal conditions. Furthermore, the recombinant nature of ELRs allows precise incorporation of bioactive peptide domains, such as the statherin - derived SNA15 sequence, which enhances mineral-binding affinity and enables controlled bio mineralization while maintaining consistent physicochemical properties and facilitating large-scale production[17,18,20, 21, 23]. Unlike purified natural enamel proteins, recombinant ELRs can be genetically designed to contain specific bioactive peptide sequences while maintaining the parent polymer's intrinsic physicochemical features. This modular design allows for the production of multifunctional biomaterials with improved mineral-binding, self-assembly, and biological functions, providing increased repeatability, batch-to-batch consistency, and flexibility for regenerative dentistry applications[21, 23, 29].

The mineral-binding characteristics of the statherin-derived SNA15 peptide appear to be the primary driving force for bio mineralization. Statherin is a naturally occurring salivary phosphoprotein that regulates calcium phosphate supersaturation within saliva and inhibits uncontrolled mineral precipitation while also demonstrating a strong affinity for hydroxyapatite surfaces[25-29]. The negatively charged aspartic acid and glutamic acid residues in the SNA15 domain form localized electrostatic binding sites that can concentrate calcium ions from the surrounding mineralization solution. This localized increase in calcium ion concentration reduces the activation energy required for heterogeneous nucleation and enhances the subsequent integration of phosphate ions into developing hydroxyapatite crystals [24-28].

Preserving the underlying crystal template is also a key factor in successful enamel regeneration. Acid etching

removes the superficial mineral layer, revealing the highly structured hydroxyapatite prism structure that persists in partially demineralized enamel. Once adsorbed on this surface, the flexible ELR network conforms closely to the exposed crystal lattice, serving as a molecular bridge between native enamel and newly deposited minerals. This tight connection promotes epitaxial crystal development, in which newly nucleated hydroxyapatite crystals adopt the crystallographic orientation of the underlying substrate rather than creating randomly oriented mineral aggregates [13-15]. The highly ordered crystal alignment found by FE-SEM and the preferential c-axis orientation confirmed by XRD in the prior research are compatible with its results on protein-guided bio mimetic enamel regeneration [13,15,28].

Studies on bio mimetic enamel remineralization have consistently found Ca/P ratios that nearly match the stoichiometric composition of biological hydroxyapatite (Ca/P = 1.67). These findings suggest that protein- and peptide-guided mineralization favours the development of structured hydroxyapatite rather than nonspecific calcium phosphate precipitates. The strong match between the regenerated mineral phase and native enamel underscores the biological relevance of these bio mimetic techniques and indicates their capacity to replicate the compositional properties required for functional enamel regeneration[13,15, 22,23].

Mechanical characterisation described in bio mimetic remineralization research indicates that restoration of enamel microstructure is associated with significant recovery of functional mechanical characteristics. Protein-guided mineralization has been demonstrated to increase nano hardness and elastic modulus, indicating greater load-bearing capacity and resistance to mechanical deformation. The creation of a highly structured hydroxyapatite crystal architecture helps to achieve these advantages by closely imitating the hierarchical structure of native enamel. In contrast to traditional resin-based restorations, which rely primarily on adhesive bonding and micromechanical retention, bio mimetic mineral layers have shown close integration with the underlying enamel substrate, with little evidence of interfacial flaws or delamination. This structural continuity is projected to minimize stress concentrations at the restoration interface, which could contribute to increased long - term durability under physiological occlusal loading [8-12,53].

One significant advantage of recombinant ELRs is their potential for cost-effective and scalable production. Although recombinant protein-based biomaterials have shown great promise in regenerative medicine, their widespread clinical application has frequently been hampered by the complexity and high cost of traditional chromatographic purification techniques like fast protein liquid chromatography (FPLC). In contrast, ELRs have reversible lower critical solution temperature (LCST) behaviour, allowing for effective purification by ITC. This chromatography-free technique takes use of the reversible thermal phase shift of ELRs, simplifying downstream processing while lowering production costs and permitting large-scale manufacturing without compromising biomaterial quality [20-23, 38].

Because ITC uses normal laboratory centrifugation and controlled temperature changes rather than specialized chromatographic equipment, it is a straightforward and cost-effective purification approach. This technique allows for the creation of high-purity recombinant proteins with minimal endo toxin levels, while lowering manufacturing complexity and facilitating scalable production for biomedical and regenerative medicine applications [22,38, 45, 63].

Endo toxin elimination is a vital step in the development of recombinant biomaterials for clinical use. Purification techniques such Triton X-114 phase separation, when paired with repeated ITC, have been demonstrated to efficiently minimize bacterial lipopolysaccharide contamination while maintaining protein integrity. Achieving minimal endo toxin levels is particularly critical for biomaterials developed for intraoral applications, because leftover bacterial endo toxins may elicit inflammatory responses, limit biocompatibility, and negatively affect tissue integration and regeneration results[45,50,63]. The capacity to produce clinical-grade purification using very modest laboratory infrastructure has the potential to considerably increase access to recombinant regenerative biomaterials, particularly in low- and middle-income nations with limited high-capital bio processing capabilities.

Pre - clinical studies indicate that ELR-based bio mimetic scaffolds have potential for enamel regeneration, with preliminary evidence indicating enhanced remineralization and reduced dentinal hypersensitivity after clinical application. These findings lend support to the use of recombinant protein-based biomaterials in minimally invasive restorative dentistry, but present clinical evidence is limited.

Limitations of Current Enamel Regeneration Strategies

Despite hopeful advances, most enamel regeneration techniques are limited to in vitro models and small-scale preclinical or clinical trials with short follow-up periods. Several problems continue to impede their integration into normal dentistry.

- Current bio mimetic techniques cannot fully replicate natural enamel production due to the absence of Ameloblast in mature enamel. Replicating the structured hierarchical arrangement of enamel prisms and inter prismatic regions is tough. Many ways result in better-organized hydroxyapatite layers, but they do not fully replicate the complicated three - dimensional architecture of natural enamel.
- Clinical translation barriers the oral environment poses ongoing obstacles such as saliva, pH fluctuations, bacterial bio films, enzymatic activity, and frequent occlusal loading. These parameters can have an impact on scaffold stability, mineralization, and long-term regeneration, however standardized clinical methods and long-term clinical evidence are rare.
- Production costs for recombinant proteins, specialty biomaterials, purification, and quality control might be high. Although technologies like inverse temperature cycling may increase scalability, more optimization is needed to achieve consistent, cost - effective, and clinically compatible large - scale manufacturing.

Overall, long - term durability, comprehensive architectural restoration, cost - effective production, and strong clinical validation remain critical for bringing enamel regeneration to regular restorative dentistry.

Future Directions

Future studies should concentrate on bigger, well-designed clinical trials with long-term follow-up to determine the safety, durability, and efficacy of ELR-based enamel regeneration.

Key emerging directions include

- 3D bio printing of enamel: Precision bio printing allows for controlled deposition of mineralizing proteins and hydroxyapatite, replicating enamel's complex hierarchical framework.
- Generating ameloblast-like cells from stem cells may address the paucity of regenerative cells in mature enamel and mimic natural amelogenesis.
- Stimuli-responsive ELR - based scaffolds with anti-bacterial, mineralizing, fluoride - releasing, or controlled

- release components can promote adaptive and multifunctional enamel regeneration.

- AI-guided regenerative design can improve protein sequences, peptide - mineral interactions, scaffold architecture, and mineralization conditions, leading to faster creation of individualized regenerative solutions.
- Clinical translation and scalability: Cost - effective manufacturing, standardized protocols, chair side delivery, and long-term clinical validation are crucial for implementing these technologies in everyday restorative dentistry.

Conclusion

Current research suggests that recombinant ELR-based bio mimetic scaffolds are a promising strategy to enamel regeneration. The incorporation of mineral-binding peptides such as SNA15 can facilitate controlled hydroxyapatite nucleation and ordered crystal development, resulting in mineral phases that resemble native enamel. Advances in recombinant protein engineering, scalable synthesis, and cost - effective purification have increased the translational potential of these biomaterials.

Unlike traditional restorative materials, which largely replace missing enamel, protein-guided bio mimetic techniques seek to mimic characteristics of natural Amelogenesis and encourage biological tissue healing. This method may help to preserve healthy tooth structure and increase the functional longevity of enamel repair. However, the current evidence is primarily preclinical, and larger clinical trials with long-term follow-up are required to confirm safety, durability, and therapeutic effectiveness. Continued advancements in smart biomaterials, protein engineering, and regenerative dentistry may eventually lead to minimally invasive biological treatments for enamel erosion, early carious lesions, and other enamel abnormalities.

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