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Composition of elastin like polypeptide–collagen composite scaffold influences *in vitro* osteogenic activity of human adipose derived stem cells

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ABSTRACT

Objective. Collagen-based scaffolds for guided bone regeneration (GBR) are continuously improved to overcome the mechanical weaknesses of collagen. We have previously demonstrated superior mechanical characteristics of the elastin-like polypeptide (ELP) reinforced collagen composites. The objectives of this research were to evaluate the efficacy of ELP-collagen composites to culture human adipose-derived stem cells (hASCs) and allow them to undergo osteogenic differentiation. We hypothesized that hASCs would show a superior osteogenic differentiation in stiffer ELP-collagen composites compared to the neat collagen hydrogels.

Methods. Composite specimens were made by varying ELP (0–18 mg/mL) and collagen (2–6 mg/mL) in a 3:1 ratio. Tensile strength, elastic modulus, and toughness were determined by uniaxial tensile testing. hASCs cultured within the composites were characterized by biochemical assays to measure cell viability, protein content, and osteogenic differentiation (alkaline phosphatase activity, osteocalcin, and Alizarin red staining). Scanning electron microscopy and energy dispersive spectroscopy were used for morphological characterization of composites.

Results. All composites were suitable for hASCs culture with viable cells over the 22-day culture period. The ELP-collagen composite with 18 mg/mL of ELP and 6 mg/mL of collagen had greater tensile strength and elastic modulus combined with higher osteogenic activity in terms of differentiation and subsequent mineralization over a period of 3 weeks compared to other compositions. The extra-cellular matrix deposits composed of calcium and phosphorous were specifically seen in the 18:6 mg/mL ELP-collagen composite.

Significance. The success of the 18:6 mg/mL ELP-collagen composite to achieve long-term, 3-dimensional culture and osteogenic differentiation indicates its potential as a GBR scaffold.

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1. Introduction

Periodontitis is the inflammation of the periodontium surrounding the tooth with progressive loss of alveolar bone resulting in complete loosening of the tooth. Guided bone regeneration (GBR) and guided tissue regeneration (GTR) are widely used techniques that regenerate the various periodontal tissues including the root cementum, periodontal ligaments, and alveolar bone, thereby decreasing the mobility of the affected tooth [1–3]. GTR uses a resorbable or non-resorbable membrane as a barrier between the epithelial and connective tissues. This membrane prevents the unwanted regrowth of the epithelial and connective tissues [3]. GBR uses a resorbable or non-resorbable scaffold to support the regeneration of the alveolar bone at a bony defect introduced by tooth extraction or disease [3]. The materials used in GBR/GTR must be biocompatible, non-immunogenic, and non-inflammatory. In addition, the GBR/GTR materials must possess adequate strength to allow proper handling for their placement at the defect site and modulus to avoid collapse under the intraoral forces. Finally, the GBR/GTR materials must degrade *in vivo* at a rate that matches the rate of the new tissue formation [3].

The current trend in periodontal tissue engineering research is focused toward bone regeneration at targeted sites by combining stem cells, scaffolds, and growth factors. Human mesenchymal stem cells (MSCs) are an attractive source of stems cells for bone regeneration as they have the capacity to regenerate and differentiate into osteogenic lineage [4]. Human adipose-derived stem cells (hASCs) have emerged as an alternative to human bone marrow MSCs (hBMSCs) due to their easy procurement from liposuction patients, rapid *in vitro* expansion, and high harvest yield [5]. hASCs have a demonstrated ability to differentiate into osteoblastic lineage *in vitro* as well as in the clinical treatment of bone defects [6–8]. Therefore, for this research, we have used hASCs to evaluate their osteogenic function in different scaffolds. For GBR applications, scaffolds act as the housing in which the osteoregenerative cells like hASCs can proliferate, differentiate, and mineralize into bone. Successful biocompatible scaffolds should possess adequate mechanical and resorption properties [3] along with suitable osteoconductive and osteoinductive properties. Unfortunately, the currently available synthetic scaffolds prove inadequate for clinical applications. For instance, the non-resorbable scaffolds require a second surgery for their removal, causing economic and physical discomfort for the patient [3]. Synthetic resorbable polymers suffer from early loss of structural and mechanical properties with poor cell response [9,10]. Natural resorbable polymers such as collagen are osteoinductive and an effective alternative to synthetic polymers due to their biocompatibility and excellent cell affinity [11–13]. However, they often suffer from rapid hydrolytic and enzymatic degradation and lack sufficient mechanical properties [14,15]. Crosslinking of collagen using chemicals [3] like glutaraldehyde and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride can overcome poor mechanical properties and antigenicity but the possibility of cytotoxicity increases [16,17]. Blending of collagen with other polymers and ceramics has shown to enhance mechanical properties and modulate specific cellular responses [18–21]. These disadvantages of both non-resorbable and resorbable materials make it clear that an "ideal" material has not been found [22] and have led to approaches that use non-crosslinked collagen composites [3].

In the design of bio-inspired bone regenerative composites with non-crosslinked collagen, elastin-like polypeptides (ELP) are under investigation as they have amino acid sequences similar to native elastin [23]. ELP, which is a soluble and recombinant form of elastin, offers an attractive solution to the problems faced by non-resorbable and resorbable synthetic materials used previously to prepare collagen-based composites. ELPs display inverse phase transition behavior which allows easy purification [20]. More importantly, ELPs possess excellent mechanical properties [24]. In the past, we prepared non-crosslinked ELP-collagen composites by utilizing the gelation behavior of collagen at 37 °C and showed their superior mechanical behavior and equivalent biocompatibility compared to neat collagen hydrogels [24,25]. We demonstrated that ELP-collagen composite scaffolds help in the attachment, proliferation, and subsequent differentiation of 3T3-E1 mouse pre-osteoblastic cells [25].

Mechanical properties such as stiffness or modulus play a crucial role in the success of a scaffold and also influence the commitment of cells to a specific lineage. Studies have shown that osteogenic differentiation is stiffness/modulus dependent in hBMSCs [26,27]. Huebsch et al. showed that both human and murine MSCs cultured in the presence of mixed osteogenic and adipogenic supplements can be made to undergo either adipogenic or osteogenic differentiation based on the matrix stiffness [28]. Though the effect of matrix stiffness has been well studied with hBMSCs, the lacuna with hASCs led to the development of this study. We aimed to study the effect of scaffold composition and stiffness on the ability of hASCs to commit to osteogenic lineage. We hypothesized that hASCs would differentiate along the osteoblastic lineage in a more rigid ELP-collagen composite compared to the neat collagen hydrogels. Hence, we investigated the efficacy of composites made with varying concentrations of ELP and collagen for hASCs culture to produce viable and differentiated osteoblast-like cells and investigated the influence of mechanical properties like elastic modulus of the composites on the osteogenic differentiation.

2. Materials and methods

2.1. Expression and purification of ELP

Preparation of ELP was performed as described elsewhere [25]. Briefly, *Escherichia coli* BLR(DE3) bacteria (Novagen, Madison, WI, USA) with synthetic gene for ELP having primary sequence of [Valine-Proline-Glycine-Valine-Glycine]$_{120}$ were cultured for 24 h at 37 °C and lysed by sonication. The inverse phase transition behavior of ELP was used for purification by three repeated cycles of solubilization in deionized water at 4 °C, followed by precipitation and centrifugation at 40 °C. The ELP solution was dialyzed and then lyophilized (Labconco Corp., Kansas City, MO, USA).