Biomimetic Mineralized Keratin Scaffold Fabricated by Rapid Electrodeposition for Potential Bone Regeneration

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Abstract

Background, the context and purpose of the study:

For bone regeneration, rapid mineralization is of particular importance in the preparation of biomimetic bone scaffolds, as for avoiding the drawbacks of prolonged mineralization which will resulted the loss of growth factors and degradation. Thus, In this research, we developed a relatively rapid mineralization approach for constructing biomimetic keratin scaffold that exhibited highly interconnected pore and natural bone mimetic calcium phosphate coating by applying an electrodeposition technique. Mineralized keratin scaffold was obtained by freeze drying followed by electrodeposition for rapid biomimetic mineralization.

Findings, the main results:

The mineral coating morphology, component, crystal structure could be controllably tailored by manipulating the deposition electrode, voltage and duration. A satisfying coating of apatite layer on keratin scaffold could be obtained within a couple of hours by electrodeposition. By increase the voltage and duration, a more favored amounts of apatite coating which dominated by HA crystal could be formed. In addition, cell regeneration assay showed that mineralized biomimetic keratin scaffold exhibited more suitable supporting platform for the proliferation and osteoblastic differentiation of MC3T3 cell over pure keratin scaffold.

Conclusions, brief summary and potential implications:

the rapid electrodeposition mineralization approach presented in this work could be highly desired for fabricating biomimetic scaffold in which biological molecules were loaded for functional bone tissue engineering applications.

1. Introduction

An emerging paradigm for bone scaffolds is to design functional scaffolds with desirable biocompatibility and biodegradability as well as present similar structure and function with natural bone tissues[1, 2]. Meanwhile, lots of studies demonstrated biomimetic scaffolds may play crucial roles in regulating cellular growth, differentiation, and bone regeneration[3]. Several approaches have been employed for the preparation of biomimetic scaffold. Among them, the in-situ mineralization method could form more similar mineral layer as natural bone, so the structure of the scaffold is closer with human bone[4]. However, most of the in-situ deposition, such as simulated body fluid method, is time-consuming and tends to lead to material degradation, strength reduction and activity loss. Therefore, it is urgent to develop high-speed and effective alternative method for realizing rapid mineralization of biomimetic scaffold[5].
Aim at developing appropriate rapid biomimetic mineralization method, electrodeposition technology have been applied as an effective way to decorate apatite coating on the surface of bone scaffold[6]. Electrochemical mineralization, a fast and efficient biomimetic tool, can modify the surface of scaffolds with structurally regular and high quality HA layers in a few hours[7, 8]. Therefore, in this project, biomimetic keratin porous scaffold was fabricated by freeze-drying with subsequently electrodeposition to form uniform apatite coating with tunable topography and chemical composition[9, 10]. The effects of different electrodes and deposition parameters on the nucleation growth and morphology of mineralization products were comprehensively studied.

2. Materials and methods

2.1. Materials

Feather is supplied by Yongfeng down products Co.Ltd., Hangzhou, China. Silk is purchased from Wensli Group Co., Ltd., Hangzhou, China. Cysteine(AR), urea(AR) is purchased from Aladdin, Shanghai, China. Acetic acid(AR), sodium hydroxide(AR) is purchased from Gaojing Fine chemical industry Co., Ltd.

2.2. Preparation of keratin scaffold

Keratin solution is obtained using reduction method according to the reported literature. Silk fibroin (SF) solution is obtained via dissolving silk fabric with 9.3M LiBr solution.

The composites are fabricated by adding SF which serve as templates into keratin solution. The mixed solution is stirred thoroughly before putting into incubator (25°C, 65% RH) to let keratin molecular interact with the templates.

2.3. Electrodeposition of the scaffold

A solution containing 0.042 mol/L CaCl$_2$ and 0.025 mol/L NH$_4$H$_2$PO$_4$ is prepared to ensure a Ca/P ratio of 1.67 and a pH of 7 in the solution as a mineralization solution placed in the electrolytic cell. Then it is fixed on the electrode sheet with conductive adhesive to serve as the cathode, the graphite electrode as the anode, and the mercuric chloride electrode to be the reference electrode.

2.4. Characterization of the scaffold

The morphology of the scaffolds and mineralized scaffolds is observed using a scanning electron microscope. In order to analyze the type, content and distribution of elements in the deposited layers on the scaffolds, an X-ray energy spectrometer (EDS) configured on the scanning electron microscope is used.

3. Results and discussion

The aim of this research is to fabricate biomimetic keratin scaffold via forming porous scaffold by freeze-drying with subsequent rapid mineralization through electrodeposition. Figure 1L schematically
illustrate the keratin porous scaffold preparation and electrodeposition process. Figure 1 demonstrates SEM and crystal structure of the mineralized keratin scaffold that deposited at 4V for 1h at different electrode. When the keratin scaffold is fixed on the stainless steel electrode, the deposited calcium phosphate salts completely cover the whole scaffold surface as shown in Fig. 1A and B. In contrast, when the keratin scaffold is mineralized on the copper electrode, the calcium phosphate salt formed is not compactly arranged and the aggregates did not have a complete and consistent shape as in Fig. 1C and D. From Fig. 1E we could observe blank areas between adjacent bulges, which indicated that the calcium phosphate salt crystals grew haphazardly and could not completely envelop the scaffold.

Figure 1.I presents the XRD pattern of the apatite deposited on the surface of keratin scaffold under different deposition electrodes. When using Cu, Ni, Ti electrode, well defined peaks at (020), (021), and (041) were clearly observed and match well with calcium phosphate dihydrate crystalline data (DCPD). While, adopting SS electrode, the XRD pattern of the deposited mineral showed some new peak at (211) that corresponding with the diffraction planes of HAp, indicating that the scaffold contained HAp in the deposited crystals only when mineralization is performed on SS electrodes. As shown in Fig. 1.K, the mineralized layer is found to be composed mainly of calcium phosphates (O, Ca and P), with a molar ratio of Ca/P of 1.41, indicating that the mineralized layer contains a variety of calcium phosphate crystals.

It can be seen from Fig. 2.A that calcium phosphate crystals grown on the surface of the scaffold at lower voltage (3V), forming a complete and uniform mineralized layer with thin thickness. A uniform and nest-like coating could be observed when the mineralization voltage increase to 4 V as shown in Fig. 2.B. When the voltage continues to rise to 5V, the scaffold is covered with multiple layers of calcium phosphate, and a large number of spherical aggregates appeared in the uppermost layer (Fig. 2.C). On the other hand, the mass of the scaffold also increased when using higher deposition voltages as shown in Fig. 2.G.

Figure 2.H presents the pattern of the mineral crystals decorated on the surface of keratin scaffold at different voltages. When the mineralization voltage is 3V, there is a clear diffraction peak of (020) crystal plane at 2θ of 11.7°, which is a typical characteristic peak of DCPD crystals. This indicates that higher deposition voltage could result in some deposited crystal being converted from DCPD to HA.

The mineralization duration is an important factor affecting the quality of the deposited layer. Effect of electrolyte duration on the mineralization of keratin scaffold was investigated by varying the mineralization time (0.5 h, 1.0 h, 1.5 h and 2.0 h) at 4V using SS electrode. Tiny granule shape of mineral crystal could be observed deposited on the scaffold from Fig. 3.A. The coatings obtained at 1.5h was plate-like, similar in shape to flower clusters as can be seen from Fig. 3.E. When the mineralization time reached 2.0 h, the calcium phosphate crystals grown into complete spheres and gathered densely on the surface of the previous layer of the deposited body.

The diffraction peaks of the (020) and (021) crystallographic planes of DCPD appeared near 2θ of 11° and 2θ of 20° when the mineralization time is short (0.5h and 1.0h). As the mineralization time is extended to 1.5h and 2h, the diffraction peaks on the crystalline surface of DCPD disappeared and the
The diffraction peak of HA at 31.9° became more pronounced. This indicates that the mineralization time is long enough to induce the transformation of crystalline DCPD to HA.

The mechanism of the electrodeposition of surface of keratin scaffold was proposed in Fig. 3K. When the deposition voltage is applied, the pH value in the vicinity around the cathode will increase and resulted the amphoteric keratin molecules becoming negatively charged. Firstly, the Ca$^{2+}$ in the electrolyte migrated to the cathode, and then the anions around the cathode, including PO$_4^{3-}$, HPO$_4^{2-}$, H$_2$PO$_4^{-}$, OH$^{-}$, combined with Ca$^{2+}$ to form various Ca–P deposits onto the surface of porous scaffolds. The nucleation and growth of calcium phosphate crystals on the template surface resulted forming different types of crystals.

### 4. Conclusion

In conclusion, we have fabricated 3D biomimetic keratin scaffold with tunable calcium phosphate deposition morphology, composition, crystalline structure by utilizing rapid electrodeposition mineralization method. The apatite deposition coating topography and chemical composition could be easily tailored by adopting different electrodeposition electrode, voltage, and duration parameters. The deposition rate of the apatite crystal could be accelerated once upon increasing either of the voltage or deposition duration. These understanding open an avenue for rapid mineralization of biomimetic scaffold for bone regeneration or other biomedical application.

### Declarations

**Ethics approval and consent to participate**: This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent for publication**: The authors approved the consent for publishing the manuscript.

**Availability of data and materials**: Will be disclosure on required.

**Competing interests**: The authors have no competing interests to declare.

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Authors' contributions: Daen Qin conceived and designed research, and conducted experiments. Daen Qin and Ruipeng Li analyzed data and wrote the manuscript. Kaili Song supervised the project, acquired funding, and reviewed the manuscript. Kaili Song, Zhicheng Yu and Aixue Dong supervised the project. All the authors read and approved the final manuscript.

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References


Figures

Figure 1

SEM images of keratin scaffolds after mineralization at different electrodes: (A, B) SS electrode, (C, D) Cu electrode, (E, F) Ni electrode, (G, H) Ti electrode; (I) XRD spectra of mineralized scaffolds; (J, K) EDS
images of and elemental analysis pure keratin and mineralized scaffold; (L) Schematic diagram showing the preparation process of keratin biomimetic scaffold

![SEM images of keratin scaffolds after mineralization at different voltages: (A, D) 3V, (B, E) 4V, (C, F) 5V; (G) Mass increase of scaffolds; (H) XRD patterns of mineralized scaffolds](image)

**Figure 2**

SEM images of keratin scaffolds after mineralization at different voltages: (A, D) 3V, (B, E) 4V, (C, F) 5V; (G) Mass increase of scaffolds; (H) XRD patterns of mineralized scaffolds
Figure 3

SEM images of keratin scaffolds after mineralization at different time lengths: (A) 0.5 h, (C) 1.0 h, (E) 1.5 h, (G) 2.0 h; (J) XRD pattern of the mineralized scaffold; (K) Schematic illustration for the hypothesized mechanism of electrodeposition

Supplementary Files

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