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Improving the osteogenesis and degradability of biomimetic hybrid materials using a combination of bioglass and collagen I

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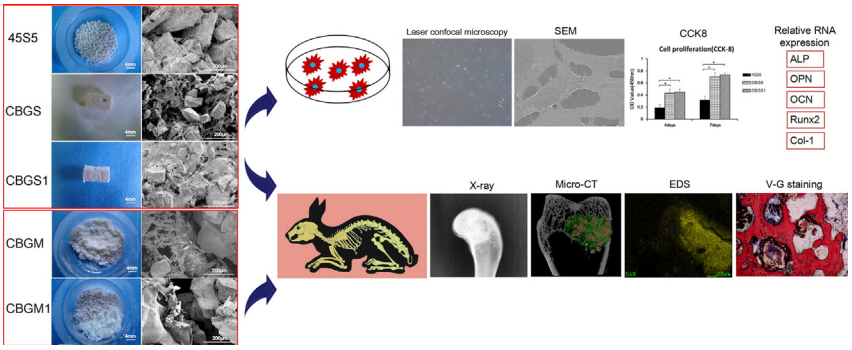
HIGHLIGHTS

- The hybrid materials of type I collagen and 45S5 are more advantageous to osteogenesis than single 45S5 granules.
- Type I collagen from bovine tendon has a better biological activity than that from bovine hide.
- Compared with morsel, the strip shape of hybrid materials is a favorable factor for osteogenesis in vivo.
- The degradation of Collagen Bioactive Glass Strips matchs the new bone formation.
- The biomimetic hybrid materials are promising bone graft candidates for clinical application.

GRAPHICAL ABSTRACT



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ABSTRACT

As an inorganic bone graft, the traditional bioglass 45S5 can offer good bioactivity for osteogenesis. Type I collagen (Col-1) can mimic the organic components of natural bone in hybrid materials. Hence, in this study, biomimetic hybrid materials [CBGM (Collagen Bioactive Glass Morsels), CBGM1, CBGS (Collagen Bioactive Glass Strips) and CBGS1] were fabricated using 45S5 granules and high purity Col-1 at a proportion of 8.5:1.5. The surface features of these materials were observed using scanning electron microscopy and element energy dispersive spectroscopic (EDS). The activity of MC3T3-E1 cells, including adhesion, proliferation and gene expression, was significantly improved in the CBGS and CBGS1 groups compared with the 45S5 group. Additionally, X-ray, micro computed tomography, EDS and histomorphology showed that these hybrid materials had significantly enhanced osteogenesis and degradation 6 and 12 weeks after implantation in rabbit femoral condylar defects compared with 45S5. CBGS and CBGS1 were significantly better than CBGM and CBGM1 in terms of osteogenesis and degradability. Consequently, with the improved osteogenesis and the faster degradation rate, the hybrid materials CBGS and CBGS1 are promising bone graft candidates for clinical application.

1. Introduction

At present, bone autografts and allografts have been widely used to treat bone defects caused by trauma [1], infection [2] or bone tumor resection [3] in clinical practice. However, these methods have some

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shortcomings [4,5], such as limited quantities of available material, complications at the donor site, and potential viral infection. Hence, research and development regarding bone graft materials, especially bioactive materials, is urgently needed in the medical profession [6,7]. Among these materials, bioactive glass, particularly 45S5 [8,9], has been widely used in clinical practice because of its excellent biocompatibility, osteogenic capability, and osteointegrative properties.

Bioactive glass 45S5 granules include five substances [10]: 46.1 mol% SiO_2, 26.9 mol% CaO, 24.4 mol% Na_2O and 2.6 mol% P_2O_5·Compared with other types of bioactive glass, it has some advantages, including an appropriate percentage of silicon, a proper Ca/P ratio, remarkable biological performance, and an active surface; thus, it offers promising potential for repairing the bone defect. Furthermore, bioactive glass 45S5 granules can provide sufficient surface area and proper clearance to promote nutrient diffusion and therefore facilitate granules' replacement with newly formed bone. The most important benefit is that bioactive glass 45S5 can be gradually degraded and hydrolyzed to release some active ions, including Ca^{2+}, Na^{+}, PO^{3-}_{4}, and OH^{-}, which can break Si—O—Si bonds, form Si(OH)4, and ultimately compound a hydroxycarbonate apatite (HCA) layer around the glass granules [11,12]. Another key point is the size of the 45S5 granules [13,14]. If the granules are too small, they can degrade too quickly, before the new bone forms, which leaves many cavities. However, excessively large granules not only affect the degradation velocity but also prevent the formation of new bone. Thus, appropriate granular size is of great concern and has a significant effect on new bone tissue [15].

Nevertheless, bioactive glass 45S5 implants can fail because their degradation rate is not consistent with crawling process of new bone tissue. In addition, bioactive glass is completely inorganic character and includes no organic substances. In comparison, collagen I (Col-1), the only type of collagen present in bone, accounts for approximately 90% of the organic component of natural bone tissue. The collagen network provides a good microenvironment for bone cells, retains calcium salt in the bone tissue and prevents the loss of calcium salt deposits. Furthermore, some scholars have concluded that if there is plenty of calcium but no collagen in bone, bone deterioration cannot be controlled [16–18]. Moreover, collagen has been made into different types of medical biomaterials and has been broadly studied in different medical fields [19–22] because of its low immunogenicity, good histocompatibility, excellent degradability and coagulation capability. Consequently, bioactive glass 45S5 granules coated with collagen I, which combine the advantages of inorganic and organic compounds, may have a greater ability to enhance bone repair.

Therefore, an understanding of how to combine bioactive glass 45S5 granules with collagen I and how to determine the appropriate proportions of each material is critical for improving the degradability and osteogenesis of bioactive glass 45S5 granules via a biomimetic process. In natural bone, the ratio of organic matter to inorganic matter is approximately 2/1, and collagen I accounts for approximately 30% of bone tissue. However, when Col-I is implanted into the body, it degrades rapidly and leaves cavities that hinder the formation of new bone tissue. Considering these factors, bioactive glass 45S5 granules should be enhanced appropriately from a bionics perspective. Furthermore, the stickiness of collagen I allows it to coat the surface of 45S5 granules without adding adhesive materials.

In this study, two types of high-purity type I collagen were extracted from bovine tendons and bovine hides via an acid process, and bioactive glass 45S5 granules were fabricated using the traditional melt-quenching route. Then, bioactive glass 45S5 granules ranging in size from 32 to 2500 μm were combined (8.5:1.5) with the high-purity Col-1 derived from bovine tendons or bovine hides. The resulting mixture was made into collagen bioactive glass morsels and collagen bioactive glass strips. Through cell experiments and animal experiments, the degradability and osteogenesis of these hybrid materials were evaluated.

2. Materials and methods

2.1. The synthesis of bioglass 45S5 granules

First, the high-purity oxides SiO_2, CaO, Na_2O and P_2O_5 were mixed evenly according to the appropriate weight proportion (45 wt% SiO_2, 24.5 wt% CaO, 24.5 wt% Na_2O and 6 wt% P_2O_5). Next, the mixture was placed in a platinum crucible and melted at high temperatures (above 1300 °C). The mixture was maintained in a melted state for 90 min. Then, the melted mixture was quenched in water and formed into bioglass frit. Finally, the bioglass frit was ground into 32- to 2500-μm bioglass granules using an agate mortar. All samples were then ultrasonically cleaned sequentially in acetone, ethyl alcohol, and deionized water for approximately 10 min for each treatment.

2.2. The extraction of Col-1

Col-1 was obtained from the fresh hide or tendon of adult bovines. The hair, fascia and subcutaneous fat were removed from the fresh bovine hide. After rinsing, the bovine hide was cut into many small pieces with a scalpel or an edge tool. The pieces were ultrasonically cleaned in normal saline and distilled water for approximately 10 min for each treatment to remove residual blood and adipose tissue. Then, the pieces were placed in a beaker, and a moderate amount of acetic acid solution was added. The acetic acid solution was stirred and shocked until it became an evenly turbid paste. At that point, NaOH solution was instilled into the supernatant of the acetic acid solution to adjust its pH value, and the sediments was removed. Then, the NaCl concentration of the supernatant was adjusted to 20%. The precipitation that was salted out using 20% NaCl was collected and placed into deionized water that was replaced three times a day for 5 days for dialysis. The resulting high-purity collagen gel samples were white flakes that were dried and then used in the subsequent experiment. This process was performed at 4 °C·The same process was used to extract Col-1 from bovine tendons.

2.3. Preparation of the hybrid materials

One manner of preparing the hybrid materials involved directly combining the dry Col-1 debris derived from bovine hide with bioactive glass 45S5 granules at a ratio of 1.5:8.5. We labeled this hybrid material Collagen Bioactive Glass Morsels (CBGM). Another preparation involved putting the wet pulpy collagen and bioactive glass 45S5 granules (1.5:8.5) in a cylindrical mold and applying modest force to produce a cylinder (diameter 6 mm, height 1.2 mm), which was then freeze-dried. These cylinders were labeled Collagen Bioactive Glass Strips (CBGS). Following the steps above, Col-1 from bovine tendon was also combined with bioactive glass 45S5 granules to produce CBGM1 and CBGS1. All samples were packed in sealed bags and sterilized through irradiation with cobalt 60.

2.4. Characterization

Field emission scanning electron microscopy (FE-SEM, S-4800, HITACHI, Japan) was used to observe the surface morphology of the hybrid materials. Element energy dispersive spectroscopic (EDS) analysis using EMAX energy software was performed to identify the chemical composition of the samples. Before the analysis, the hybrid materials were sputtered with platinum (Pt) using a same sputtering instrument to improve the surface conductivity.

2.5. In vitro cell studies

2.5.1. Cell culture

Mouse preosteoblast cells from the MC3T3-E1 cell line were cultured in complete medium (L-DMEM medium supplemented with 10% fetal