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Research Article

The Effect of Adding Chondroadherin to an Injectable Ceramic Bone Substitute on Bone Ingrowth

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Abstract In an ideal synthetic bone substitute, the material should be partly or totally resorbed with time and replaced by host bone. A combination of calcium-phosphate (CaP) and calcium sulphate (CaS) will lead to resorption of the sulphate and allow bone ingrowth. Chondroadherin (CHAD) as a noncollagenous cartilage protein may play an important role in the regulation of chondrocyte growth and proliferation. Adding CHAD to a bone substitute may play a role in speeding up the bone integration. To explore the release of CHAD, pellets of material were placed in PBS for up to 14 days. Supernatant absorbance was measured in a UV-Vis spectrophotometer. Results showed a steady release of CHAD most notable especially within the first hour. To explore bone integration with the combined material, pellets were implanted in a bone harvest chamber bilaterally in rabbit tibia. After 2 and 3 weeks, tibiae were harvested and prepared for histology evaluation. Results showed that the number of osteoclast cells was higher in specimens containing CHAD than those without, but there is no significant difference. A trend of increasing new bone area and new bone contacting implanted material was found at 2 weeks and at 3 weeks with CHAD. Changes of dosage and material composition should be considered in further studies.

Keywords injectable bone substitute material; Chondroadherin; alpha-tri-calcium phosphate; calcium sulphate; release

1 Introduction

The development of biphasic injectable bone substitute material, in particular α-tri-CaP/CaS, has provided a source of treatment for osseous defects originating from fractures and bone disease. It exhibits excellent biocompatibity, osteoinduction, and conductivity. Furthermore, it provides the possibility of incorporating additional biocompatible substances which may facilitate or enhance the bio-integration and/or bone formation process.

Chondroadherin (CHAD) is a peptide which was first isolated by Larsson [1] from bovine cartilage. It is described as a noncollagenous cartilage protein found in the territorial matrix of articular cartilage [2] with cell binding properties [5]. It has been suggested that chondroadherin may play an important role in the regulation of chondrocyte growth and proliferation [5]. This is being due to its high expression as a cell binding protein in the dynamic region of maturing articular cartilage. Mizuno [3] concluded that chondroadherin may play a role in maintaining bone cells on the collagen matrices of bone.

Such findings provide an interesting basis to explore chondroadherin's impact in the bone formation system. In order to know the effect of incorporating CHAD into injectable bone substitute materials on bone growth, the release of CHAD was investigated in *in vitro* laboratory environment. Meanwhile, bone integration to the materials and osteoclast formation around the materials were studied in bone harvest chamber in rabbit tibia.

2 Materials and methods

2.1 Material preparation

Alpha tricalcium phosphate (α-TCP) 80 wt% and α-calcium sulphate hemihydrate ($CaSO_4.^{1/2}H_2O$, CaS) 20 wt% were prepared as a bone substitute. Calcium pyrophosphate and calcium carbonate were mixed in the stoichiometrically correct proportions and heated to $1325^{\circ}C$ and then quenched rapidly in air. The materials were sterilized by gamma irradiation. The CHAD peptide used in the preliminary preparation steps and subsequent testing was obtained in the dry freeze form. In order to maintain consistency and comparability, all CHAD samples were sourced from the same batch.

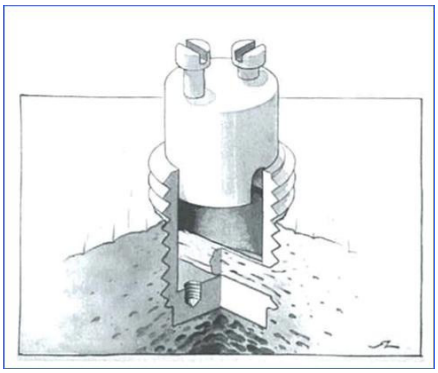

Figure 1: Schematic drawing of the bone harvest chamber imbedded in rabbit tibia [4].

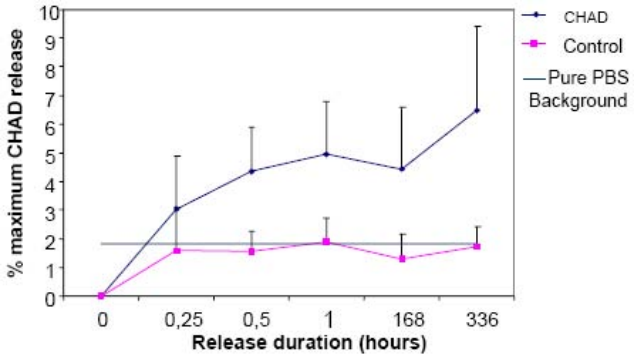

Figure 2: UV absorbance as a percentage of total CHAD release from 5 pellets.

2.2 Pellet preparation

The bone substitute was mixed with 2.5% Na_2HPO_4 solution at a liquid powder ratio of 0.36 mL g^{-1} as a control. The CHAD peptide (100 μg) was dissolved in 72 μL of 2.5% Na_2HPO_4. The CaP/CaS (200 mg) was mixed in the solution. It contained 0.5 μg CHAD per mg of CaP/CaS. The CaP/CaS was injected into a mold (1 mm thick and 1 mm diameter). The materials were dried for approximately 3 hours at room temperature then refrigerated until testing for a maximum of 4 days. The material was removed from the mold as pellets using a thin, flat ended stainless steel rod placed directly on top of a pellet perpendicular to the plate, and a gentle force applied until the pellet dropped out. If a pellet's shape deteriorated during extraction, it was discarded. The control and CHAD containing pellets were produced, respectively, in the mold and were used in both *in vitro* and *in vivo* studies.

2.3 *In-vitro* study

5 pellets were placed in each vial containing 100 μL PBS. 6 samples were used for each time point, 15, 30 minutes, 1 hour, 7 days, and 14 days. After each required duration, samples were centrifuged at 10,000 rpm for 10 minutes. 80 μL of supernatant was removed, and absorbance was measured in a UV-Vis spectrophotometer at a wavelength of 280 nm. The solution was then placed back into the same vial until the next time point. Release of CHAD over time and visual observations of pellet stability over time were recorded. This value was then divided by absorbance reading mean values by quantity of chondroadherin present in pellets and then multiplied by 100 to obtain the percent of total chondroadherin released at any given time.

2.4 *In-vivo* study

Six, adult, lop-eared rabbits (4.0 to 5.5 kg) were used. Under general anesthesia, bone harvest chambers (BHCs) were implanted bilaterally in the proximal tibiae of each rabbit (Figure 1). The chamber contained a core with a $1 \times 1 \times 5$ mm groove which allowed direct contact and subsequent ingrowth of bone tissue. Pellets with or without CHAD were placed into the center of the channel in the chamber. One chamber received CaP/CaS as a control, and the other chamber received CaP/CaS with CHAD. Six paired samples from each time period were harvested at 2 and 3 weeks intervals. Specimens were fixed in 2.5% glutaraldehyde and were dehydrated in alcohol and embedded in Technouit 7110 solution. The samples were then cut into sections $6 \sim 7$ micrometres in thickness and stained by Goldner and Trap. New bone area (NBA), bone contact percentage (BCP), thickness of new bone (TNB), and osteoclast count (OcC) were analyzed by histology and histomorphology under a microscope. Percent of new bone area was a ratio of new bone area/total tissue area and multiplied by 100. Percent of new bone contact was calculated by dividing the length of new bone in contact with the implanted material by the circumference of all implanted materials, multiplied by 100. Thickness of new bone was then obtained by direct division of new bone area divided by total length of new bone. Total osteoclast count required division of the total number of osteoclast cells by the total bone area.

3 Results and discussion

In vitro. For samples containing CHAD, a distinct increasing trend was noted from 15 minutes to the first hour (Figure 2). The release then increased steadily over the following two weeks period, reaching a maximum average release (6.47%) at two weeks. Visual qualitative inspection of the pellets after a period of two weeks showed very little decomposition. Leading to confirmation of the hypothesis that dissolution of pellets is minimal within two weeks. Thus, the source of CHAD is primarily the result of surface dissolution.
In vivo. No significant difference was found in NBA, BCP, and TNB between control and CHAD specimens in both periods, but results showed a trend of increasing NBA, BCP

at 2 weeks and TNB at 3 weeks with CHAD (Figures 3, 4, 5, and 6). A clear trend was noted, OcC was higher in specimens containing CHAD (Figure 7) than those without, although there is no significant difference (Figure 8). This increase may result from active bone formation during the first 2 weeks. In this period, osteoblasts may stimulate osteoclast formation giving rise to fast bone integration.

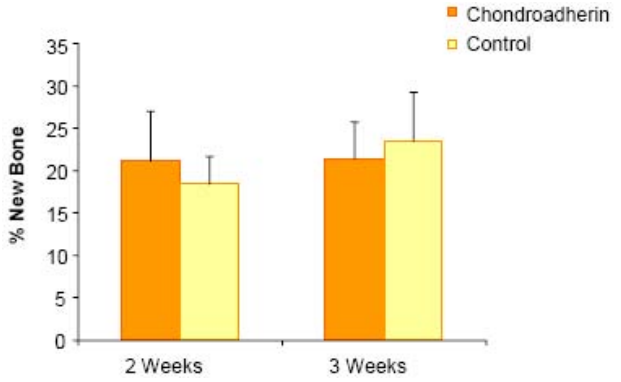

Figure 3: Percentage of new bone growth.

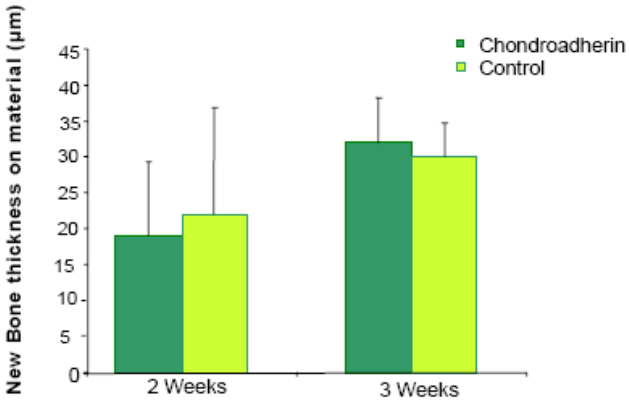

Figure 4: Thickness of new bone contact.

4 Conclusions

CHAD is released into solution over time, especially within the first hour. This was made clear through a notable increasing trend over a two week period with respect to control samples. CHAD seems speed up early bone integration at 2 weeks and stimulates bone thickness as bone remodeling at 3 weeks. Though, overall, it seems that with the current sample set and size, these trends are not sufficiently high enough to imply statistical significance. The trends, however, were certainly of interest. Changes of dosage and material composition should be considered in further studies.

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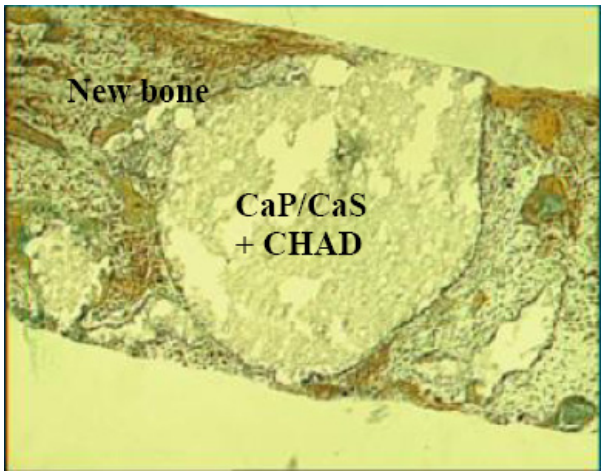

Figure 5: Histological section of CHAD treated sample at 2 weeks (×4).

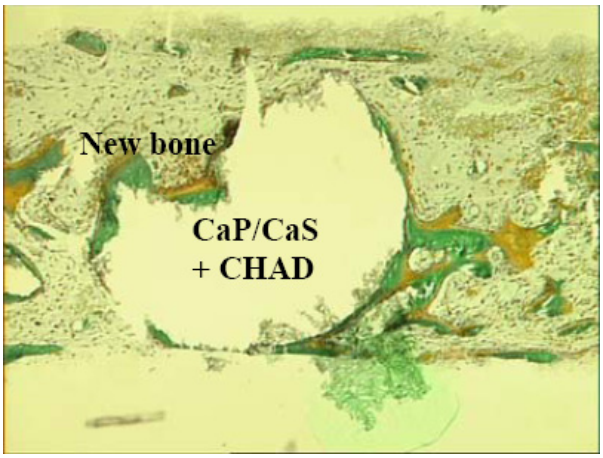

Figure 6: Histological section of CHAD treated sample at 3 weeks (×4).

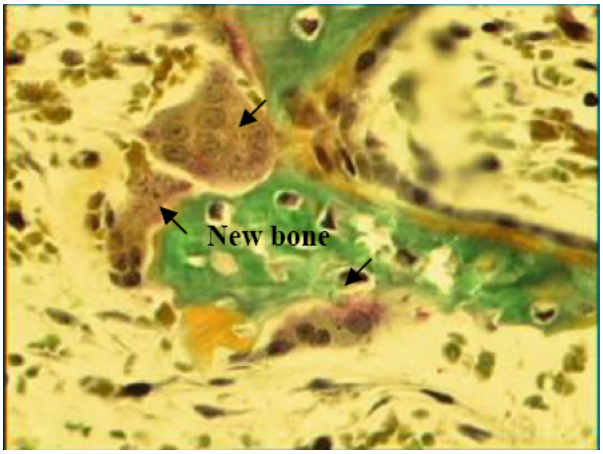

Figure 7: Histological section of CHAD treated samples at 3 weeks. Arrows show osteoclasts activity surrounding newly calcified bone (×20).

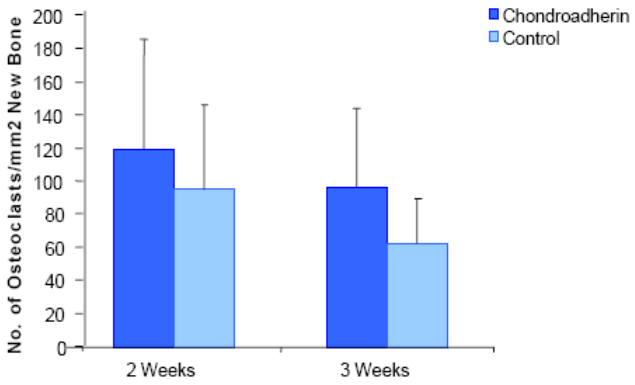

Figure 8: Number of osteoclasts present per mm^2 of new bone following bilateral implantation of the biomaterial.

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