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Short Communication

In vitro biocompatibility of dextrin: the addition of a low concentration of dextrin in the medium promotes the cell activity of L929 mouse fibroblasts

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Abstract

To develop a bone substitute with shape-generating properties, we focused our attention on dextrin, which has a low viscosity. After considering methods of evaluation for research and development, we started by using cells that are widely used for safe biological evaluations in the field of dentistry and conducted *in vitro* evaluations. In this experiment, we variously added concentrations of 0.1, 1.0 and 10 mmol/l of dextrin to a culture medium in order to examine the effects on L929 mouse fibroblasts *in vitro*. As a result, the proliferative activity of the L929 cells was promoted during the culture period as the concentration of added dextrin became lower, and in particular, the 0.1 and 1 mmol/l addition group showed higher values than those of the control group. From the above results, it was revealed that the addition of a low concentration of dextrin in a medium promotes the cell proliferative activity.

Keywords: biocompatibility; cell proliferation; dextrin; IC_{50}; L929

1. Introduction

Calcium phosphate materials are frequently used for the reconstruction of bone defects in the field of dentistry, and hydroxyapatite and such similar substances are clinically applied (Uchida et al., 1990; Beck-Coon et al., 1991; Cooke, 1992). The development of a new composite material with a bone substitute and a polymeric material has been attempted in order to compensate. Until now, studies that combine polymeric materials such as fibrin (Matras, 1982), dextran (Nagase et al., 1991), chitosan (Kawakami et al., 1992) and collagen (Blumenthal et al., 1986) as a vehicle with a bone substitute and fill it have been reported. Natural polymeric materials are not frequently used in clinical setting because it is necessary to ensure safety, including antigenicity, when using protein with a biologic origin. Based on the above description, we focused our attention on dextrin, which is a polysaccharide with a good metabolic process and a low viscosity, as a filler that may be an alternative to these materials. To have good biocompatibility, materials must meet such conditions as the absence of not only tissue irritation and cytotoxicity, but also antigenicity and allergic responses. For materials that have been used as dental materials in the past, various *in vitro* evaluations have been conducted as part of basic research (Jacob et al., 2003; Qiang et al., 2008).

If dextrin is administered or implanted as an implantation material *in vivo*, biocompatibility with the tissues is essential. Therefore, in this experiment, each concentration of dextrin in the medium was used to examine how dextrin affects the cell proliferation of mouse fibroblasts (L929), based on its application to bone defects.

2. Materials and methods

2.1. Observation of the surface texture of dextrin

Dextrin (Pine Fiber®; Matsutani Chemical Industry Co., Ltd), which has an average molecular weight of 2000, was used as the experimental material. The surface texture was observed via a scanning electron microscope (JSM-5900LV; JEOL). The accelerating voltage was 10 kV.

2.2. Cell proliferation test for L929

L929 (ATCC CCL 1; Dainippon Sumitomo Pharma Co., Ltd) derived from mouse fibroblasts was used for the cells. The medium was adjusted by adding 5 wt% FBS (fetal bovine serum; Invitrogen Japan KK) and 100 U/ml penicillin–100 μg/ml streptomycin (Invitrogen Japan KK) to MEM (minimum essential medium; Invitrogen Japan KK). Dextrin used in this study had been already sterilized after processing. The experimental group consisted of those in which 0.1, 1.0 and 10 mmol/l of dextrin had each been added to the medium, and the control group used only the medium. These different media underwent filtration (22 μm) after each concentration of dextrin was supplemented to the medium.

The culture was adjusted so that L929 would be 2×10^4 cells/ml, 400 μl each of which was seeded in a 24-well microplate. Each group was cultured under conditions of 37°C and 5% CO_2 concentration over various culture periods of 24, 48, 72 and 96 h. Each medium was replaced after 2 days.

CCK-8 (Cell Counting Kit-8; Dojindo Laboratories) was used to measure the number of living cells (Ishiyama et al., 1997; Tominaga et al., 1999). Specifically, the medium in each well that

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Abbreviations: CCK-8, Cell Counting Kit-8; IC_{50}, 50% cell inhibitory concentration values.

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had been cultured for 24, 48, 72 and 96 h was replaced with a new medium, 40 μl of CCK-8 was added to each well, 100 μl each of which was transferred to a 96-well microplate after letting it stand for 1 h and the absorbance (O.D. 450 nm) was measured with a microplate reader (MPR-A4i; Tosoh Corporation). A certain number of cells (2×10^4–17×10^4 cells/ml) was prepared for calculating the number of living cells, which was obtained from an analytical curve created by measuring the absorbance ($n=5$).

2.3. IC_{50} (50% cell inhibitory concentration values) of L929

We then further added a group in which 100 mmol/l dextrin was added to the medium to 0.1, 1.0 and 10 mmol/l addition groups at 24 h after culturing, and the IC_{50} was thus obtained from the concentration–cell survival rate curve ($n=5$).

2.4. Observation of the cell morphology of L929

Cell morphology was observed via a phase-contrast microscope (IX71; Olympus Corporation) at 48 and 96 h after culturing.

2.5. Statistical processing

To evaluate the number of living cells, one-factor ANOVA (analysis of variance) followed by Tukey's *post hoc* test was performed. *P*-values of less than 0.05 were considered to be statistically significant. All values are expressed as the mean ± S.D.

3. Results

3.1. Observation of the surface texture of dextrin

A 500-magnified SEM (scanning electron microscopy) image of the surface of the dextrin specimen is shown (Figure 1). The particle of dextrin presented a spherical or elliptical shape, with a particle size of 50 to 100 μm.

3.2. The cell proliferation test for L929

The results of the cell proliferation test, in which dextrin was added to the medium, are shown (Figure 2). All of the experimental groups and control underwent cell proliferation during the culture period. The 0.1 mmol/l addition group showed a higher value than did the control after 72 h of culturing, which thus represents a significant difference. The 1.0 mmol/l addition group showed a higher value than did the control group at 96 h after culturing, which thus represents a significant difference. The 10 mmol/l addition group showed a significantly lower value than did the control after 48 h of culturing.

3.3. IC_{50} of L929

The cell survival rate of L929 relative to the dextrin concentration at 24 h after culturing is shown (Figure 3). The IC_{50} value was 72 mmol/l according to the concentration–cell survival rate curve.

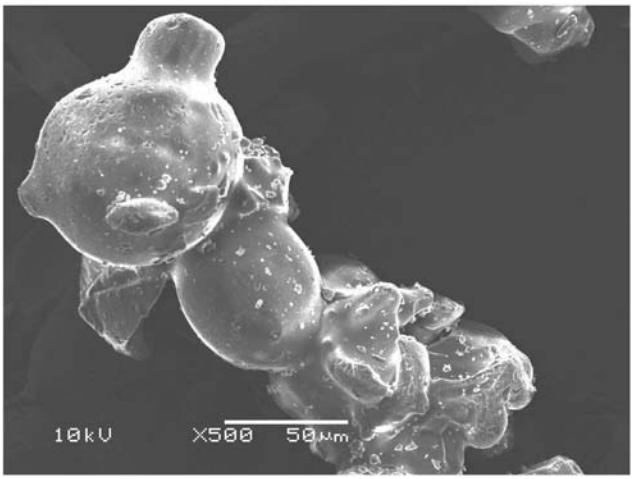

Figure 1 Surface texture of dextrin
The particle of dextrin presented a spherical or elliptical shape, with a particle size of 50 to 100 μm.

3.4. Observation of the cell morphology of L929

The cell morphology at 48 h after culturing took on a spindle shape in all of the groups. All of the groups showed polygonal and spindle shapes by 96 h after culturing, with mature cells and several cells undergoing cell division being observed (Figure 4).

4. Discussion

Dextrin is a generic name for an intermediate product that decomposes starch, producing maltose, which can be obtained by processing starch with enzymes, acid, heat and so on. It is typical for the structure to have α-1,2 or α-1,3 glycoside bonding in addition to α-1,4 and α-1,6 glycoside bonding. It is believed that new bonding was

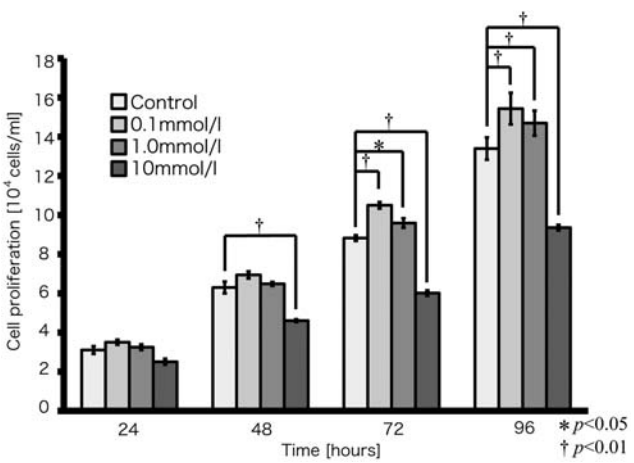

Figure 2 Cell proliferation test for L929
All of the experimental groups and control underwent cell proliferation during the culture period. The proliferative activity of L929 was promoted as the dextrin concentration became lower.

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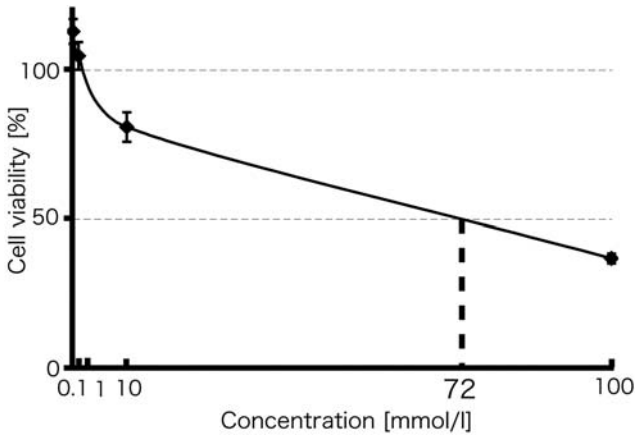

Figure 3 IC_{50} of L929
The IC_{50} value was 72 mmol/l according to the concentration–cell survival rate curve.

produced because a transfer or retrosynthesis reaction occurred at the same time as hydrolysis. Dextrin is water soluble, has an easily adjustable viscosity and it can be metabolized in the body via hydrolysis. Dextrin is classified as a low-molecular dietary fibre, and dextrin with a plasma-extending effect has also recently been developed. In addition, it is used as a vehicle for antibiotics, has a low viscosity, is stable against heat and acid and also has excellent preserving properties. In addition to drugs, it has been applied in various fields such as those of food, cosmetics, paper manufacturing, fibre and adhesives. As described above, because dextrin has excellent physiological effects and chemical properties, we believe it can be clinically used as an effective implantation material. Because the dextrin that was used in this experiment has lower antigenicity compared with the implantation materials that have been reported thus far, and it can be decomposed into monosaccharide in the metabolic process, it is believed that it has less damaging effects *in vivo* (Kato et al., 1995).

The cell proliferation test using L929 is described in ISO 7405 and ISO 10993-5, and in the field of dentistry, the effects on materials and drugs are evaluated using the same cell (Taira et al., 2000; Jinno et al., 2006). In this experiment, as well, we conducted a cell proliferation test based on the standard for a similar biological safety evaluation. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay that was used for calculating the number of living cells is widely used for cell proliferation tests as an alternative method to the [^{3}H] thymidine uptake test because WST-8 reagent is reduced by NADPH or $NADP^+$, produced by dehydrogenase in the cells via metabolic activity, and the quantity of formazans produced is proportional to the number of living cells (Ishiyama et al., 1997; Tominaga et al., 1999). It is believed that the cell morphology of L929 is significantly less damaging to the cells because all of the groups had mature cells, and a few cells undergoing cell division were observed at 96 h after culturing. In addition, from the results of the cell proliferation test, the proliferative activity of L929 was promoted as the dextrin concentration became lower. Specifically, it was indicated that the addition of 0.1 mmol/l of dextrin to the medium, which is a low concentration of dextrin, is useful for the metabolic activities of cells. We believe that this addition of a low concentration of dextrin therefore provided important intracellular nutrition to L929.

On the other hand, the medium showed strongly increased viscosity after the addition of 10 mmol/l of dextrin especially. As a result, the cell proliferation was thus assumed to have been suppressed because the nature of the medium had been physically changed due to the addition of a high concentration of the dextrin. Furthermore, in the initial stages of culturing, cell proliferation was suppressed compared with the control. However, because dextrin is metabolized via hydrolysis *in vivo*, we believe that even if the proliferation had been suppressed in the same way that it had been *in vitro*, then the level of suppression would be minimal.

This research is comprised of basic research regarding dextrin, which has a low viscosity, and our final goal is to develop a bone

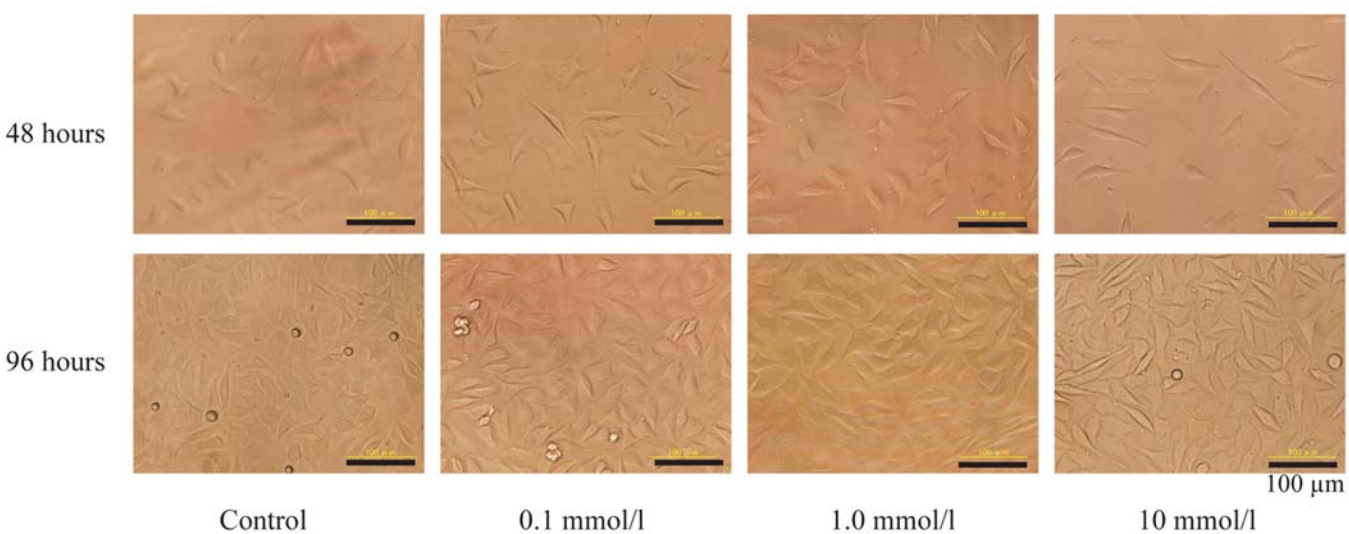

Figure 4 Cell morphology of L929
The cell morphology at 48 h after culturing took on a spindle shape in all of the groups. All of the groups showed polygonal and spindle shapes by 96 h after culturing, with mature cells and several cells undergoing cell division being observed.

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substitute with shape-generating properties and which can be used in combination with various ceramics. When conducting future *in vivo* experiments based on the insights obtained from these results, it will be necessary to provide formability to the ceramic materials and discover the optimal concentration in order to prevent the suppression of cell proliferation.

When the concentration of dextrin added to the culture solution was low, L929 exhibited a good proliferative activity. This suggests that dextrin exhibited a good biocompatibility *in vitro*, and it is therefore considered to be useful as a vehicle for use with bone substitutes.

Author contribution

Takafumi Asai was the main researcher and corresponding author of this study. Tatsuhide Hayashi was the experimental adviser of this study. Kenjiro Kuroki, Masashi Okano and Takashi Kiriyama were responsible for the L929 cell culture and statistical processing. Tatsushi Kawai was in charge of the overall investigation and was the experimental adviser of this study.

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