Bovine Colostrum Increases Proliferation of Canine Skin Fibroblasts¹,²

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EXPANDED ABSTRACT

KEY WORDS: bovine colostrum • dog • fibroblasts • proliferation

Bovine colostrum (BC) is a milk secreted during the first few days after calving and is a rich source of bioactive components such as immunoglobulins, insulin-like growth factor (I, II) (IGF-I-II), transforming growth factor-β (TGF-β), platelet-derived growth factor, epidermal growth factor, tumor necrosis factor, basic fibroblast growth factor, vascular endothelial growth factor, and telomerase (1–3).

The importance of colostrum for the health of young animals has been known for a long time (1–4), but other systemic effects in adult humans fed BC orally have also been demonstrated, such as increased salivary IgA and plasma IGF-I (5), reduced symptoms of upper respiratory tract infection in humans (6), and changes in body composition of human athletes (7).

Specific growth factors have been studied in different cultured cells, such as cementoblasts (8), chondrocytes (9), fibroblasts (10), and myoblasts (11) to evaluate improved regenerative therapies for periodontal tissues, osteoarthritis, and wound healing.

The aim of this study was to evaluate the effects of a skim freeze-dried BC source (Colexan, Colostrum Technologies GmbH) rich in bioactive components (immunoglobulins, natural growth factors, and hormones), as an in vitro stimulator of canine skin fibroblast proliferation and activity. This in vitro model could be used as a model for wound repair efficiency and periodontal tissue repair for topical applications.

MATERIALS AND METHODS

Skin samples

Skin samples were taken from fresh abdominal skin obtained from surgeries in 2 different public kennels in Barcelona (Arrabassada and Granollers). The skin (n = 4) was always healthy skin from dogs between 2 and 12 y old.

Fibroblast isolation and culture

Cultures of normal canine dermal fibroblasts were established from canine skin samples taken from 4 dogs. The skin was well shaven and cleaned with 70% EtOH/Betadine before the start of cell isolation. All the fat tissue and blood vessels were removed from the skin with scissors, and then it was washed with 0.02 mol/L sodium phosphate buffer with 0.15 mol/L sodium chloride, pH adjusted to 7.4, without calcium or magnesium (PBS⁻).

Skin cells were enzymatically dispersed from skin biopsies. Briefly, washed skin was chopped into 1-mm³ fragments and incubated for 140 min in 15 mL/g skin of Dulbecco’s Modified Eagle Medium (DMEM) (Gibco, Invitrogen) containing 30 mg collagenase, 12 mg pronase, 1.5 mg DNAse (all from Sigma) supplemented with 450 mg bovine albumin and antibiotics (100 U penicillin, 100 µg streptomycin) (Gibco, Invitrogen). After digestion, cutaneous cells were washed with PBS⁻, and their viability was assessed. Cells were grown in DMEM supplemented with 5% fetal calf serum and antibiotics (all from Gibco) in 100-mm culture dishes for the initial plating and passages. Fibroblasts grew in a humidified atmosphere at 37°C with 5% CO₂ for 2 d. Cells were attached, and the medium was changed twice a week until the first passage was necessary. Fibroblasts were used at passages 2–5.

Bovine colostrum source

A freeze-dried skim BC source (Colexan, Colostrum Technologies GmbH) with 85–90% total protein (85% IgG), <1% fat, 1–5% lactose, 5–7% ash, and natural growth factors and hormones (IGF-II, 2–5.3 µg/mL; TGF-β, 0.1–0.4 µg/mL; cortisol, 170 µg/g; prolactin, 4 ng/g; estradiol, 1.98 ng/g; testosterone, 33.6 ng/g; insulin, 1.98 µU/mL; and osteocalcin, 702 ng/g) was used.

MTT cell proliferation assay

Activity that stimulated cell growth was assayed with the addition of various concentrations of BC in a 96-well microplate, and the plate...
COLOSTRUM EFFECTS ON DOG SKIN FIBROBLAST CULTURE

Effect on fibroblast proliferation of different concentrations of bovine colostrum added to a fibroblast culture for 24 or 48 h

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OD Culture time 24 h</th>
<th>Relative activity, %</th>
<th>OD Culture time 48 h</th>
<th>Relative activity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (−)</td>
<td>0.343 ± 0.009</td>
<td>100</td>
<td>0.447 ± 0.007</td>
<td>100</td>
</tr>
<tr>
<td>Control (+) SCF, 100 ng/mL</td>
<td>0.460 ± 0.020</td>
<td>134</td>
<td>0.643 ± 0.020</td>
<td>144</td>
</tr>
<tr>
<td>Bovine colostrum:</td>
<td></td>
<td></td>
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<tr>
<td>1 mg/mL</td>
<td>0.452 ± 0.002</td>
<td>132</td>
<td>0.591 ± 0.010</td>
<td>132</td>
</tr>
<tr>
<td>0.3 mg/mL</td>
<td>0.410 ± 0.002</td>
<td>120</td>
<td>0.501 ± 0.004</td>
<td>112</td>
</tr>
<tr>
<td>0.1 mg/mL</td>
<td>0.408 ± 0.011</td>
<td>119</td>
<td>0.477 ± 0.014</td>
<td>107</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Cell proliferation was estimated by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which was first described by Mosmann in 1983 (12). This assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals, which are largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of the cells by the addition of a detergent (dimethyl formamide) results in the liberation of the crystals, which are solubilized. The number of surviving cells is directly proportional to the level of the formazan product created. The color can then be quantified using a simple colorimetric assay. The results can be read on a multwell scanning spectrophotometer (ELISA reader).

Briefly, cells were plated at a density of 10,000 cells per well with the different concentrations of colostrums to be tested (0.1, 0.3, and 1 mg/mL by dry weight, respectively) or stem cell factor (SCF) (100 ng/mL, Amgen) as positive controls because it promotes cellular growth. Negative controls (without any kind of factor that stimulates the cells) were plated with the same water volumes as colostrums in the culture medium. Cells were incubated for 24 and 48 h at 37°C in 5% CO₂ humidified atmosphere, and 10 µL of MTT were added to 100 µL of medium per well and incubated for 4 h until purple precipitate was visible. Fifty microliters of dimethylformamide was added and incubated at 37°C in 5% CO₂ humidified atmosphere in the dark for 18 h. Finally, the absorbance was read at 570 nm. The number of surviving cells was directly proportional to the level of the formazan product created.

Moreover, canine fibroblast growth was still significantly stimulated in a dose-dependent manner by the 2 highest concentrations (stimulation of 12 and 32% related to negative control by 0.3 and 1 mg/mL, respectively, P < 0.05) after 48 h of incubation.

The results of this trial showed that freeze-dried skim BC significantly (P < 0.05) stimulated dog fibroblasts at any dose used (0.1–1 mg/mL) at 24 h culture incubation, and the effect remained significant at 48 h for the dose of 0.3 and 1 mg/mL. This activity of BC has already been shown on NIH 3T3 cells (13) and in human fibroblasts (14). Hironaka et al. (13) observed a stimulation of NIH 3T3 cell growth only with the cream fraction of bovine colostrum and not with skim colostrum, but in our case, skimmed BC was able to stimulate cell growth at different doses.

Further investigation would be useful to identify which components of BC are efficacious in improving canine fibroblast growth.

The results of this trial showed that freeze-dried skim BC maintains activity of the bioactive substances that promote the cellular growth, suggesting potential proliferative actions by direct contact with target cells. Further studies are needed to evaluate not only local effects but any systemic effect of an oral supplementation of BC for regenerative therapies of different tissues.

### Statistical analysis
Results are presented as values of absorbance at 570 nm in optical density units (OD). Differences of absorbance were assessed using Student’s t test. Means in a row without a common superscript letter differ, P < 0.05.

### Results and Discussion
Isolated canine fibroblasts were incubated with BC at 3 different concentrations (0.1, 0.3, and 1 mg/mL) for 24 and 48 h. Canine fibroblast growth, measured by the absorbance of the different cultures (Table 1), was significantly increased in a dose-dependent manner by the 3 different concentrations after 24 h of incubation (19 to 32% related to negative control, P < 0.05).

### Literature Cited