Effect of selected wound antiseptics on adult articular cartilage (bovine sesamoid bone) in the presence of *Escherichia coli* and *Staphylococcus aureus*

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Running title

Effect of antiseptics on cartilage

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Abbreviations

bsb: bovine sesamoid bone(s); PG: proteoglycan(s)

Abstract

The iodophore Betaisodona® [0.5 % (v/v) PVP-I], the biguanide polihexanide (PHMB) [0.005 % (v/v)], and the bispyridinamine Octenidine (Oct) [0.005 % (v/v)] effectively removed an innoculum of 10^8-10^9 cfu of *Escherichia coli (E. coli) or Staphylococcus aureus (S. aureus)* from bovine sesamoid bones (bsb) within 2 h . Subsequently, bsb were cultured for 7 d and then biosynthetically labelled with ^{35}S-sulfate for a period of 24 h. Proteoglycans (PG) of culture media and cartilage matrix of bsb were examined. The antiseptic treatment did not result in an increase of catabolism of PG. The treatment with the iodophore stimulates the incorporation of ^{35}S-sulfate into PG, whereas that of Oct was toxic. The PHMB treatment was both tolerated and effective only when it was used at low concentration (0.005%).

This *in vitro* study clearly demonstrates that irrigation of cartilage with an antiseptic should be limited to the lowest concentration and treatment time compatible with antiseptic function. Iodophores have no negative feedback on cartilage metabolism, moreover, they stimulate chondrocytes *in vitro*. Cationic antiseptics are not suited as irrigating solutions.

Introduction

Bacteria may invade joints, trigger an inflammatory response and thus cause joint injury. Most cases of septic arthritis are caused by a small group of bacteria including staphylococci, streptococci, gonococci, and Gram-negative bacteria [30]. Bacteria may reach the joint by various routes, most commonly by hematogenous spread, though direct inoculation entry by a penetrating injury, arthrocentesis, arthroscopy or total joint arthroplasty can also occur. Finally, an infection may originate from a soft-tissue infection or periarticular osteomyelitis [13,17]. To prevent contamination after trauma or for treatment of bacterial joint infection both drainage of the joint and application of antimicrobial drugs topically or systemically should be carried out as early as possible [2,12,16,28]. Direct application of antiseptics with an immediate microbicidal effect against microorganisms may also be applied to the open joint in the emergency room [21]. However, it is important to characterise potential adverse effects of these agents on tissue function by *in vitro* and *in vivo* studies using experimental animals. For this purpose, a suitable *in vitro* model will be needed for both to estimate the effective concentration required for killing bacteria in the presence of articular cartilage and to characterize the biochemical response of chondrocytes to this treatment. Bovine sesamoid bones (bsb) from metacarpophalangeal joint have been employed for the investigation of chondrocyte metabolism in response to various stimuli [19,20,27,34,36,37] and were used in the present study. Final concentrations 0.5 % of the PVP-I, 0.005 % of Oct and 0.005 % of PHMB were the minimal effective concentration for killing 10^8-10^9 colony forming units/ml of *E. coli* or *S. aureus* in 1 h in the presence of bsb [26]. To characterize a possible toxic effect on chondrocytes in the anatomically intact articular cartilage as a result of this treatment, bsb were metabolically labelled with ^{35}S-sulfate after 7 d in culture. The present investigation demonstrates that toxic effects of the polycationic antiseptics PHMB and Oct depends on the applied concentration of the active agent. In contrast, the iodophore treatment stimulates the ^{35}S -sulfate incorporation into proteoglycans (PG).

Materials and Methods

*Bsb and culture medium***.** The medial bsb from both metacapophalangeal joints of 2-year-old cows were dissected aseptically within 2 h of slaughter, and adhering soft tissue was carefully removed. Each set of four medial bsb from the same cow made up one experimental group. One medial bsb from the right joint and one from the left were used as controls. Each concentration of antiseptic was studied in quadruplicate experiments. Each bsb was rinsed with 2 x 10 ml PBS to remove adhering synovial fluid and was subsequently immersed in 9 ml Ham`s F12 culture medium, supplemented with 0.1 % (w/v) globulin-free bovine serum albumin (A-4161, Sigma, Germany), 0.2 mM sodium sulfate, and 10 ng/ml IGF-I (I-3769, Sigma, Germany) in a sterile 50-ml polypropylene test tube (30 x 115 mm, Greiner, Germany)[36,37].

Microorganisms. The investigation was carried out with *S. aureus* (ATCC 6538) and *E. coli* (ATCC 11229). Trypticase-soy-broth used as diluent and the trypticase-soy-agar culture medium were purchased from Oxoid (Unipath GmbH, Wesel, Germany).

Antiseptics. The following substances and commercially available antiseptics were tested:

Betaisodona® Solution (Mundipharma, Limburg, Germany): 100 ml solution contains 10 g poly(1-vinyl-2-pyrrolidone-)iodine-complex (PVP-I), with a content of 1.1 % available iodine. The stock solution was diluted with phosphate buffered saline (PBS) resulting in 1.0 % and 0.5 % (w/v) PVP-I.

Lavasept® concentrate (Fresenius AG, Bad Homburg, Germany) contains 20 g polihexanide (polyhexamethylene biguanide, PHMB) with an average molecular weight of 2,800 and 1 g macrogolum 4000 in 100 ml aqueous solution. 0.01 % and 0.005 % (w/v) PHMB were prepared by dilution with PBS.

Octenisept® solution (Schülke & Mayr, Norderstedt, Germany) contains 0.1 g Octenidin dihydrochloride (Oct) and 2 g phenoxyethanol in 100 ml aqueous solution. 0.01 % and 0.005 % Oct was prepared by dilution with PBS.

All solutions were prepared under sterile conditions and used within 2 h. PBS was purchased from Biochrom AG (L1815), Berlin, Germany.

Combinations, culture and labelling. The test combinations were prepared by adding 1 ml bacterial broth culture containing 10^8 - 10^9 cfu (colony forming units)/ml to one bsb in 9 ml Ham`s F12 culture medium. After mixing, 10 ml antiseptic or 10 ml PBS as control was added. The combinations were cultured for 1 h at 37 °C with occasional agitation and the bsb were then transferred into the respective antiseptic solution or PBS (control) and incubated for another hour at 37°C with occasional shaking. After the antiseptic treatment the bsb were rinsed five times with 10 ml PBS each rinse being for 5 min. Subsequently, each bsb was immersed in 10 ml Ham`s F-12 culture medium and cultured at 37 °C. Media was changed every other day. The cultures were labelled on Day 7 for 20-24 h with 1.85 MBq/ml ^{35}S-sulfate (ICN Biomedicals, Germany) in culture medium. All incubations were carried out at 37 °C in a humidified atmosphere with 5 % CO_2 in air.

Isolation of cartilage biopsies. After ^{35}S-labelling bsb were rinsed with 5 x 10 ml PBS each for 5 min to remove unincorporated label. Cartilage plugs of 2.8 mm in diameter were punched out using a biopsy needle and the cartilage plugs were carefully removed from the underlying bone with the aid of a sharp scalpel. Five cartilage plugs were removed and the wet weight of each was determined immediately. Each plug was then transferred to a 1.5 ml Eppendorf tube.

Extraction of cartilage biopsies. To remove unincorporated radioactive sulfate, each cartilage plug was initially extracted by shaking on a rocking table for 4 h at room temperature in 0.5 ml 0.15 M sodium acetate buffer, pH 6.8, containing the protease inhibitors [(0.1 M 6-amino hexanoic acid, 10 mM benzamidine hydrochloride, 5 mM N-ethylmaleimide, and 10 mM ethylene diamine tetra acetic acid (EDTA)] . The second extraction involved shaking for 24 hours in 0.5 ml of fresh buffer. Under these conditions mainly diffusible PG (PG-iso: digestion products and non-aggregating PG) were extracted. The subsequent extraction of the cartilage discs was carried out in 0.5 ml 4 M guanidinium chlorid, 50 mM sodium acetate, pH 5.8, containing the protease inhibitors as described as above for 72 h on a rocking table . Under these dissociative conditions mainly aggregating PG (PG-diss) were extracted. For the isolation of PG-DTT which may be bound over disulfide bridges in the matrix the double preextracted cartilage plugs were treated with 0.5 ml 4 M guanidinium chloride, 0.05 M sodium acetate, pH 6.8 containing 10 mM dithiothreitol but no protease inhibitors [35] for 72 h on a rocking table. Finally, the stepwise extracted cartilage plugs were digested in 0.5 ml 0.15 M Tris.HCl buffer, pH 8.0, containing 1 mg/ml pronase (Serva, Germany) for 24-48 h at 56 °C. Under these conditions the cartilage is completely digested and all remaining PG (PG-residue) are converted into glycosaminoglycan chains.

Analyses of PG. Aliquots of culture media, extracts and residue were analysed for total sulfated glycosaminoglycans (GAGs) and for ^{35}S-GAGs by the dimethylmethylene blue method [23]. Qualitative characterization of PG by agarose gel electrophoresis and autoradiography using Kodak X-OMAT, XAR-2-film (Sigma, Germany) were performed as described [23,24].

Autoradiography of cartilage slices. Pieces of 8 x 5 x 3 mm from each bsb consisting of cartilage- and bone-structure were placed immediately into 50 ml-Falcon-tubes, which contained 5 ml of Lilli`s fixative with 10 % (w/v) cetylpyridiniumchloride (Serva, Germany), and were fixed and decalcified by shaking on a rocking table for 1 week at room temperature. The pieces were removed and washed essentially as described [39]. This procedure allows for maximal retention of PG. The specimens were subsequently embedded in Tissue-Tek O.C.T.-compound (Miles lab, Elkhart, IN, USA) und sections were cut at 10 µm perpendicular to the cartilage surface with L.O.T. cryostat (Shandon-Bright, Germany) at –20 °C.

Finally, air-dried cryocut slices fixed on microscope slides were coated with Hypercoat LM-1 emulsion (RPN 40, Amersham, Germany) using the dipping technique. After an exposure of 4 weeks the sections were developed and fixed essentially as described by the manufacturer and mounted in Dako Glycergel (Dako Corpoation, Carpinteria, CA, USA).

Results

Solutions of 0.5 % PVP-I, 0.005 % Oct, and 0.005 % PHMB are effective in killing 10^8 – 10^9 cfu/ml *E. coli* or *S. aureus* in the presence of bsb without reduction of synthetic activity [26]. In order to determine a potential toxic effect of this antiseptic treatment of bacterial inoculation on chondrocytes in articular cartilage, the bsb were cultured over 7 days and released matrix molecules were determined in the media. The amounts of released PG during culture of bsb were similar or lower to that of the control, indicating that there was no significant catabolism induced as a result of the various antiseptic treatment (Table 1). In contrast, in the case of cationic antiseptics there were significantly lower amounts of PG released during the culture period compared to that in the control experiments.

The stepwise extraction procedure differentiates between fragments of matrix constituents and intact less soluble constituents. Except at high concentrations of the antiseptics the percentage of total PG extracted was comparable in all test groups. At the highest concentrations somewhat stronger incorporation of PG into the cartilage matrix results which resulted in a higher percentage of PG present in the residual fraction (Table 2).

Because of possible differences in the total PG content of medial bsb from the left and from the right joint of the same animal, each set of experiment was carried out using one bsb from one joint as control sample and the other as test sample. There were no significant differences between experimental groups treated with antiseptic and the control bsb treated with PBS alone in terms of total PG content. Furthermore the antiseptic treatment did not reduce the total PG content of articular cartilage plugs recovered after culture. Using 0.5 % PVP-I for killing 10^8-10^9 cfu/ml test organisms the incorporation rate of radioactive sulfate into the PG of articular cartilage was increased by a factor of more than 2 compared to that of the control after 7 d culture of bsb. On the other hand, killing *S. aureus* with 0.005 % PHMB treatment caused no reduction in the ^{35}S-sulfate incorporation into, whereas killing *E. coli* with the same antiseptic decreased incorporation to approximately 40 % of that in the control. Moreover, using 0.005 % Oct for killing both test organisms the incorporation of radioactive sulfate was reduced to about 10 % of that estimated in the control bsb (Tables 3).

Usage of 0.01 % PHMB and 0.01 % Oct results in an incorporation of ^{35}S-sulfate into articular cartilage of approximately 30 % and 2 %, respectively. Only the 1.0 % PVP-I-treatment was tolerated by articular cartilage of bsb. The incorporation of radioactive sulfate was approximately 20 % higher than that of the control (Table 4).

Autoradiographic investigations of the main PG fraction (PG-diss), electrophoretically separated by using 1.2 % agarose gels, revealed that chondroitin sulphate rich aggregans are the principal newly synthesized PG in the control and PVP-I experimental group (Fig. 1). The increase of ^{35}S-sulfate incorporation into these PG is restricted to the middle layers or zones of cartilage (Fig. 2).

Discussion

Most effects of different antimicrobial irrigation fluids on articular cartilage were determined after the application of clinically relevant concentrations, which were derived from clinical experience in other fields [1,5,6,9,10,11,14]. In all cases, an inhibitory effect on cartilage vitality, was demonstrated which may be the result of prolonged exposure or of excessively high concentrations. Indeed, using shorter exposure times and lower concentrations for irrigating cartilage does reduce the negative effects of antiseptics [15,18,29,38]. Therefore, in a preliminary study [26] we determined the minimal microbicidal concentration and the effective incubation time for three selected antiseptics in killing *S. aureus* and *E. coli* in the presence of articular cartilage. 0.5 % PVP-I, 0.005 % PHMB, and 0.005% Oct were effective in killing the bacteria without causing increased degradation of cartilage or PG during 7 d of culture. Residual *E. coli* or *S. aureus* would destroy cartilage in vitro within 48 h causing degradation and loss of PG [32]. In this study test microorganisms and their products such as lipopolysaccharides (LPS) in the case of *E. coli* or the *Staphylococcal* PG-releasing factor [33,22], were thoroughly removed after antiseptic treatment by rinsing the cartilage in PBS. The amounts of released PG from the cartilage of bsb during 7 d of culture after treatment with 0.005 % PHMB and Oct were significant lower compared with the control and PVP-I treatment. This may be caused by superficial binding of these cationic agents which may prevent diffusion of PG and its fragments. Stimulating and inhibiting effects of the tested antiseptic agents on cartilage metabolism of bsb were estimated by ^{35}S-sulfate incorporation. The inhibition of PG-synthesis using Oct and PHMB was expected, because it is known that these polycationic active agents in both preparations bind to anionic groups, especially to sulfate groups of PG in the cartilage matrix [25]. However, after Oct-treatment, the incorporation rate of ^{35}S-sulfate in cartilage was reduced more strongly than for PHMB. Additionally, after completely removing *S. aureus* inoculation in the presence of bsb with a final concentration of 0.005% PHMB, the amount of newly synthesized PG was equal to that in the control. The differences between Oct and PHMB may be explained by different interaction of the active agent with bacteria and/or matrix and cells of cartilage. PHMB may have a more pronounced attraction to the negatively charged bacterial surface [8] than to matrix constituents or cells of cartilage, which may be the opposite for Oct. PHMB-treatment may be useful in removing bacteria from cartilage, if a defined predetermined concentration is applied, but this is not the case for Oct.

In this study, PVP-I-treatment produces no negative effects in cartilage. Moreover, there was a stimulation of ^{35}S-sulfate-incorporation. This was unexpected because it has been shown that dilute PVP-I solutions (0.01- 1.0 %) inhibit skin fibroblast growth [3]. In addition, irrigating intact rat articular cartilage with undiluted Betadine, which is comparable to Betaisodona®, resulted in a significant inhibition of 55 % in ^{35}S-sulfate incorporation compared with the control [9]. In these studies, the contact time between antiseptic solution and cells may have been too long or the concentration of antiseptic may have been too high. On the other hand, it has been demonstrated that dilute PVP-I is rapidly bactericidal [4] and does not induce cartilage damage [6] if applied for a short period. The stimulating effect of 0.5 % PVP-I seen in this study is supported by the fact that murine fibroblasts (L929, ATCC CCL 1) in cell culture treated for 30 min with a final concentration of 0.0125-0.025 % PVP-I showed a stimulation of cell growth of between 15-30 % after 72 h (our unpublished results). PVP-I`s capacity to destroy bacteria in vitro in the presence of cartilage without negative impact on cartilage metabolism, indicates that its investigation in a suitable *in vivo* model, such as the rabbit model of septic arthritis [30] is warranted. A procedure which allowed for the rapid and effective removal of bacteria from the joint cavity without inhibiting cartilage metabolism could be of great benefit in clinical practice. Nevertheless, our results clearly demonstrates, that irrigation of cartilage with an antiseptic, should be carried out only with the lowest antiseptic concentration and the shortest time compatible with efficient removal of the bacterial contamination.

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Figure legends

FIG. 1. PG banding pattern after agarose gel electrophoresis (part A, toluidine blue staining) and autoradiography of the corresponding electropherogram (part B) of ^{35}S-PG, extracted with 4 M guanidinium chloride from articular cartilage of bsb treated with 0,5% PVP-I or 0.005% PHMB (L) compared to the control (C). The migration position of the respective PG standard (1 = CS-rich aggrecan, 2 = KS-rich aggrecan, 3 = small PG) and of chondroitin sulfate standard (4) is indicated by arrows.

FIG. 2. Tissue-Tek embedded cryocut sections of bsb consisting of cartilage- and bone structure cut at 10 µm and the resulting autoradiography of newly synthesized ^{35}S-labelled PG in the cartilage layers of the control (left) and the sample treated with 0.5% PVP-I (right); sbs = sesamoid bone surface, sbc = sesamoid bone cartilage.

Table 1

Total amounts of PG released from the bsb during 7 d of culture after antiseptic treatment (2 x 1 h) with PVP-I, PHMB and Oct for killing *E. coli* or *S. aureus* compared to the control

Test combinations	Mean of released PG [mg CS-equivalents[+)]/bsb ± S.D.]
Control[++)] (n = 36)	8.0 ± 2.5
0.5 % PVP-I / E. coli (n = 4)	8.2 ± 2.0
0.5 % PVP-I / Staph (n = 4)	7.6 ± 1.9
1.0 % PVP-I / E. coli / Staph (n = 4)	7.3 ± 4.1
0.005 % PHMB / E. coli (n = 4)	4.9 ± 1,3[*]
0.005 % PHMB / Staph (n = 4)	5.7 ± 1.6
0.01 % PHMB / E. coli / Staph (n = 4)	5.4 ± 3.1
0.005 % Oct / E. coli (n = 4)	3.3 ± 0.8[**]
0.005 % Oct / Staph (n = 4)	4.0 ± 1.6[*]
0.01 % Oct / E. coli / Staph (n = 4)	3.7 ± 1.9[*]

[+)] CS = chondroitin A-sulfate from swine rib cartilage (Sigma, C7571) was used as standard

[++)] PBS treatment of bsb without bacterial inoculation were added into one group;

[*] significant different from the control ($p < 0.05$)

[**] significant different from the control ($p < 0.01$)

Table 2

Relative amounts (mean and range) of PG stepwise extracted from bovine articular cartilage plugs under isoosmolaric (PG-iso), dissociating (PG-diss) and reducing conditions (PG-DTT) as well as non-extractable residual PG (PG-rest) after antiseptic treatment with PVP-I, PHMB and Oct for killing *E. coli* or *S. aureus* compared to the control

Test combinations	1. Extraction (0.15M Na-acetate buffer + PI, pH 6.8) [%]	2. Extraction (4M Gu-HCl, 0.05M Na-acetate + PI, pH 5.8) [%]	3. Extraction (4M Gu-HCl, 0.05M Na-acetate + 10mM DTT, pH 6.8) [%]	Non-extractable residue (0.15M TRIS-HCl, pH 8 + 1mg/ml Pronase) [%]
Control	3 (0.7 – 4.2)	63 (51.7 – 78.5)	12 (6.0 – 18.4)	22 (10.2 - 33.5)
0.5 % PVP-I / *E. coli*	5 (4.8 – 5.5)	63 (58.5 – 67.0)	14 (9.0 – 22.0)	18 (14.5 – 20.5)
0.5 % PVP-I / *S. aureus*	5 (4.6 – 5.4)	63 (61.8 – 66.2)	14 (9.3 – 18.9)	18 (14.7 – 19.1)
1.0 % PVP-I / *E. coli*	3 (2.4 – 3.0)	56 (49.1 – 63.7)	13 (10.2 – 18.4)	28 (23.4 – 30.0)
1.0 % PVP-I / *S. aureus*	2 (2.1 – 2.4)	58 (52.1 – 62.1)	11 (9.3 – 12.4)	29 (25.3 – 33.1)
0.005 % PHMB / *E. coli*	3 (2.7 – 3.0)	68 (64.6 – 72.9)	11 (9.0 – 12.0)	18 (15.1 – 20.5)
0.005 % PHMB /*S .aureus*	2 (2.0 – 2.3)	67 (62.1 – 71.4)	10 (7.9 – 14.3)	21 (18.6 – 23.5)
0.010 % PHMB / *E. coli*	3 (2.1 – 2.9)	58 (53.1 – 64.6)	13 (12.1 – 14.2)	27 (20.4 – 30.6)
0.010 % PHMB / *S.aureus*	4 (3.9 – 4.4)	55 (50.0 – 62.1)	13 (9.8 – 14.3)	29 (19.2 – 34.2)
0.005 % Oct / *E. coli*	3 (2.5 – 3.6)	66 (64,6 – 67.4)	13 (11.1 – 14.1)	18 (16.9 – 21.8)
0.005 % Oct / *S.aureus*	2 (1.5 – 2.1)	64 (62,7 – 65.0)	12 (6.9 – 14.5)	23 (18.8 – 28.8)
0.010 % Oct/ *E. coli*	1 (0.7 – 1.3)	64 (59.3 – 67.5)	11 (8.7 – 13.7)	24 (21.8 – 26.3)
0.010 % Oct/ *S.aureus*	1 (0.4 – 0.9)	66 (64.9 – 67.5)	8 (7.4 – 8.6)	25 (23.5 – 26.8)

Table 3

Total PG content (mean ± S.D.) of cartilage plugs and ^{35}S-sulfate incorporation in 24 h into PG in bsb cultured for 7 days after killing *E. coli* or *S. aureus* using 0.5 % PVP-I, 0.005 % PHMB, and 0.005 % Oct, respectively.

Test combinations	Animal No.		Total PG [µg CS-equivalents/mg wet weight cartilage plug]		^{35}S-sulfate incorporation rate [cpm/mg wet weight cartilage plug]		^{35}S-sulfate uptake [%] of control	
	E.coli	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*	*S.aureus*
Control-1	1r	7r[§]	60.06 ± 4.38	61.96 ± 16.73	1274.0 ± 194.9	1080.1 ± 233.5		
0.5 % PVP-I (1)	1r	7r[§]	57.44 ± 10.48	64.02 ± 4.69	3483.0 ± 608.8[*]	2206.6 ± 402.6[*]	273	204
Control-2	1l	7l[§]	56.88 ± 9.31	54.60 ± 4.54	1237.3 ± 259.4	1164.2 ± 269.4		
0.5 % PVP-I (2)	1l	7l[§]	59.54 ± 10.49	54.45 ± 9.86	2922.9 ± 307.0[*]	2531.5 ± 339.5[*]	236	217
Control-3	2r	8r[§]	54.47 ± 3.96	56.42 ± 14.65	1173,7 ± 198,1	1020.6 ± 220.6		
0.5 % PVP-I (3)	2r	8r[§]	51.20 ± 9.20	57.84 ± 2.89	3097.9 ± 494.8[*]	1987.4 ± 389.8[*]	264	195
Control-4	2l	8l[§]	52.26 ± 7.92	48.82 ± 7.60	1139.0 ± 242.1	1043.0 ± 207.5		
0.5 % PVP-I (4)	2l	8l[§]	52.32 ± 8.99	49.55 ± 5.66	2574.0 ± 320.5[*]	2283.7 ± 215.2[*]	226	219
Control-5	3r[§]	9r	66.02 ± 3.24	74.91 ± 7.74	2142.1 ± 253.5	1840.5 ± 101.6		
0.005 % PHMB (1)	3r[§]	9r	68.03 ± 5.01	71.30 ± 4.63	997.9 ± 226.2[*]	2093.6 ± 281.1	47	113
Control-6	3l[§]	9l	61.71 ± 4.76	77.52 ± 7.37	2449.3 ± 247.3	1938.2 ± 365.9		
0.005 % PHMB (2)	3l[§]	9l	62.09 ± 8.39	74.97 ± 4.54	880.5 ± 125.8[*]	1955.2 ± 331.1	36	100
Control-7	4r	10r	56.29 ± 2.48	66.94 ± 7.07	1838.1 ± 173.0	1690.1 ± 121.7		
0.005 % PHMB (3)	4r	10r	58.09 ± 4.20	64.01 ± 4.65	852.5 ± 197.0[*]	1881.2 ± 271.7	46	111
Control-8	4l	10l	51.20 ± 3.66	69.69 ± 3.97	2029.9 ± 173.0	1736.5 ± 262.2		
0.005 % PHMB (4)	4l	10l	55.65 ± 8.09	66.86 ± 3.05	788.9 ± 123.3[*]	1742.0 ± 276.7	39	100
Control-9	5r[§]	11r[§]	49.33 ± 5.01	44.91 ± 4.05	2849.7 ± 392.3	1863.7 ± 308.4		
0.005 % Oct (1)	5r[§]	11r[§]	48.98 ± 4.69	46.35 ± 14.11	218.1 ± 44.6[*]	112.4 ± 50.0[*]	8	6
Control-10	5l[§]	11l[§]	41.89 ± 3.27	38.39 ± 4.03	2566.9 ± 283.3	2042.7 ± 305.4		
0.005 % Oct (2)	5l[§]	11l[§]	41.84 ± 5.30	37.99 ± 3.15	216.5 ± 72.2[*]	172.5 ± 53.1[*]	8	8
Control-11	6r[§]	12r[§]	54.74 ± 4.07	50.79 ± 5.87	2412.2 ± 354.4	2349.2 ± 143.8		
0.005 % Oct (3)	6r[§]	12r[§]	55.48 ± 4.58	48.72 ± 12.61	286.8 ± 97.1[*]	218.8 ± 105.6[*]	12	9
Control-12	6l[§]	12l[§]	46.14 ± 3.08	43.22 ± 4.78	1702.9 ± 299.8	2254.1 ± 464.2		
0.005 % Oct (4)	6l[§]	12l[§]	45.25 ± 6.22	41.56 ± 4.30	134.5 ± 48.7[*]	187.3 ± 52.4[*]	8	8

[§] total PG content of bsb cartilage from the left and the right hand side were significant different ($p < 0.05$)

[*] incorporation rate of ^{35}S-sulfate into the cartilage PG of experimental bsb was significant different from the control ($p < 0.001$)

Table 4
Total PG content (mean ± S.D.) of cartilage plugs and ^{35}S-sulfate incorporation rate in 24 h into PG in bsb cultured for 7 days after killing *E. coli* or *S. aureus* using 1.0 % PVP-I, 0.01 % PHMB, and 0.01 % Oct, respectively.

Test combinations	Animal No.	Total PG [µg CS-equivalents/mg wet weight cartilage plug]	^{35}S-sulfate incorpoation rate [cpm/mg wet weight cartilage plug]	^{35}S-sulfate uptake [%] of control
Control-1	13r§	37.97 ± 6.92	2543.8 ± 529.1	
E. coli/PVP-I (1)	13r§	36.17 ± 0.72	2814.7 ± 511.7	110
Control-2	13l§	45.92 ± 3.02	2191.1 ± 411.2	
E. coli/PVP-I (2)	13l§	43.91 ± 4.81	3004.5 ± 338.4*	137
Control-3	14r	61.67 ± 6.22	2452.1 ± 333.7	
S. aureus/PVP-I (1)	14r	58.87 ± 4.66	2733.2 ± 264.5	111
Control-4	14l	66.37 ± 3.84	2185.3 ± 375.6	
S. aureus/PVP-I (2)	14l	63.55 ± 2.68	2877.1 ± 475.4*	132
Control-5	15r	43.20 ± 10.11	2144.6 ± 586.7	
E. coli/PHMB (1)	15r	36.88 ± 2.98	738.4 ± 118.4*	34
Control-6	15l	46.46 ± 6.08	2272.0 ± 638.6	
E. coli/PHMB (2)	15l	39.37 ± 5.32	799.1 ± 110.5*	35
Control-7	16r§	42.48 ± 3.11	2002.1 ± 503.9	
S. aureus/PHMB (1)	16r§	43.30 ± 4.29	546.5 ± 141.3*	27
Control-8	16l§	35.45 ± 5.16	2598.9 ± 285.8	
S. aureus/PHMB (2)	16l§	36.02 ± 2.43	671.3 ± 85.2*	26
Control-9	17r	64.36 ± 4.82	2814.0 ± 287.4	
E. coli/Oct (1)	17r	61.09 ± 7.47	53.7 ± 8.7*	2
Control-10	17l	62.09 ± 16.62	2589.1 ± 628.4	
E. coli/Oct (2)	17l	64.91 ± 7.90	44.9 ± 6.8*	2
Control-11	18r	49.39 ± 7.22	2422.7 ± 498.9	
S. aureus/Oct (1)	18r	46.50 ± 4.29	65.8 ± 19.4*	3
Control-12	18l	45.07 ± 3.33	2542.2 ± 709.5	
S. aureus/Oct (2)	18l	47.57 ± 5.11	59.2 ± 15.5*	2

§ total PG content of bsb cartilage from the left and the right hand side were significant different ($p < 0.05$)
* incorporation rate of ^{35}S-sulfate into the cartilage PG of experimental bsb was significant different from the control ($p < 0.05$)