Collagenous scaffolds supplemented with hyaluronic acid and chondroitin sulfate used for wound fibroblast and embryonic nerve cell culture

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Conflict of interest

none declared

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Abstract

Background. Tissue engineering is a strategy aimed at improving the regeneration of injured tissues.

Objectives. The aim of the present study was to determine whether a tri-copolymer composed of cross-linked collagen, chondroitin sulfate and hyaluronic acid (Col + CS + HA) provides a better environment for fibroblast and embryonic nerve cell culture than a collagenous scaffold (Col).

Material and methods. The porosity of each of the matrices was characterized with a scanning electron microscope. Fibroblasts were isolated from rat wound granulation tissue (polypropylene net implanted subcutaneously). Embryonic nerve cells were obtained from the brains of rat embryos. The cells were applied to scaffolds and then stained with bisbenzimidazole to calculate cell entrapment within the material. The metabolic activity of the cells cultured within the scaffolds was tested using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Results. The Col scaffolds had a homogeneously porous structure with a pore diameter of 50 µm for 70% of pores. The pore diameter in the tri-copolymer (Col + HA + CS) ranged from 24 to 160 µm (95% of total pore volume). Four times more cells (fibroblasts and embryonic nerve cells) were trapped within the superficial part of the collagenous scaffold than that of the tri-copolymer. On the third day of culture the metabolic activity of the fibroblasts within the 2 tested scaffolds was significantly higher than in the control conditions (cell culture on a laminin-coated surface). Also, the embryonic nerve cells demonstrated increased metabolic activity in Col + CS + HA scaffolds than the Col scaffolds.

Conclusions. Both fibroblasts and embryonic nerve cells could be seeded within the 2 tested scaffolds. Both the scaffolds provide good conditions for fibroblast culture. However, the Col + CS + HA tri-copolymer is preferable for embryonic nerve cell engineering.

Key words: collagen, fibroblasts in vitro, embryonic nerve cells

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Large wounds lead to disturbances of homeostasis. Immediately following an injury, hemostasis and inflammation are induced. These processes are followed by cell migration and proliferation phases, after which fibroblasts begin the synthesis of an extracellular matrix. Together, these processes lead to scar formation that rearranges the continuity of the tissue.¹ Large wounds heal very slowly due to the large space that needs to be covered by the scar. Although limited results can be obtained by treating large wounds with conventional methods, tissue engineering provides a chance to achieve better outcomes.

Cell engineering is a new strategy developed to regenerate nerve tissues. The engineered cells are supported by a biodegradable scaffold that not only forms a mechanical strut, but also may exert a regulatory influence on cell migration, proliferation and extracellular matrix synthesis. Thus, the implants obtained not only close the wound but also stimulate the self-repair or self-regeneration of the wound.²

The favorable physical and chemical properties of collagenous (Col) scaffolds for regenerative medicine have been described by Pietrucha et al.^{3–6} The Col materials create a supportive microenvironment for fibroblasts, osteoblasts and embryonic nerve cells.^{2,6–8} Wound fibroblasts are thought to enter the scaffold from the margins of the wound and participate in scar formation. Collagen may regulate the function of cells in the wound by binding to the integrin subfamily ($\alpha 1\beta 2$, $\alpha 2\beta 2$, $\alpha 10\beta 2$ and $\alpha 11\beta 2$), which would influence granulation tissue remodeling, angiogenesis, fibroblast adhesion and myofibroblast differentiation in the wound.⁹

Hyaluronic acid (HA) is a polysaccharide composed of glucuronic acid and N-acetylglucosamine. A scaffold containing hyaluronic acid promotes wound repair in rats, accelerates wound closure, supports re-epithelialization and angiogenesis in mice, and stimulates fibroblasts to release growth factors in rats.^{10–11} Chondroitin sulfate (CS), composed of glucuronic acid and N-acetylgalactosamine, was found to promote fibrotic scar formation in an injury of the central nervous system.¹² CS is negatively charged due to the presence of SO_4^{2-} and COO^- moieties in the molecule, and this negative polarity allows the adhesion of growth factors and cells. In addition, CS influences the migration of cells.¹³

Some studies report that scaffolds composed of collagen, HA and CS have beneficial effects when used for skin or cartilage regeneration.^{14,15} The materials were seeded with skin fibroblasts or adipose-derived stem cells.¹⁴ Li et al. constructed a scaffold composed of collagen, hyaluronic acid and chondroitin-6-sulfate for nucleus pulposus tissue engineering. The copolymer was characterized by a highly porous structure, hydrophilic properties, good mechanical stability, low immunogenicity and good biocompatibility.¹⁶

The aim of the present study was to determine whether supplementation of a Col scaffold with CS and HA can in-

fluence the biological properties necessary for fibroblast and embryonic nerve cell culture, so that the scaffold providing the best environment for a given purpose can be selected. A previous study found that a scaffold composed of collagen cross-linked with CS constitutes a good environment for embryonic nerve cells.⁶ A novel element of the present study was that it investigated whether a collagen tri-copolymer with the addition of HA and CS (Col + HA + CS) can enhance cultures not only of embryonic nerve cells, but also wound fibroblasts. In this way, the study determined the usefulness of this tri-copolymer for tissue engineering.

Material and methods

The animals

The experiments were performed on 7 male and 9 female Wistar rats weighing 230 ± 40 g. The animals were kept on a 12 : 12 light/dark cycle (from 7:00) and were given free access to standard rat feed (Bakutil, Radzyń Podlaski, Poland) and tap water ad libitum. The study was approved by the Local Ethics Committee in Łódź, Poland.

Wound model

A 4 cm skin incision was made in the left lumbar region of each anesthetized rat and 3×2 cm polypropylene mesh was inserted subcutaneously. The incision was closed with 4 silk sutures. Four weeks after the wound was made, the implant, overgrown by connective tissue, was removed under aseptic conditions.¹⁷

Fibroblast isolation and culture

The removed implants containing granulation tissue were stored in RPMI 1640 medium (Gibco, Paisley, UK) containing gentamicin (25 $\mu\text{g}/\text{mL}$) and fungizone (2.5 $\mu\text{g}/\text{mL}$). After mincing, the tissue was incubated in 0.1% collagenase solution (37°C, 5% CO_2) for 40 min. After centrifugation (5 min, 1000 rpm), the samples pellets were washed with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, gentamicin and fungizone (Gibco, Paisley, UK), then centrifuged and plated on dishes. The non-adherent cells were washed out after 2 h. The cells were cultured in a CO_2 incubator at 37°C in a 100% humidified atmosphere of 5% CO_2 and 95% air. Six-well plates (TPP Techno Plastic Products AG, Trasadingen, Switzerland) were used for cell culture.

After reaching confluence, the cells were trypsinized and passaged to new flasks. New cultures were set up at an initial cell density of $8 \times 10^3/\text{cm}^2$. Bürker's chamber was used for cell counting. Necrotic cells were stained using trypan blue elimination assays. After the second passage, the experiments were carried out in DMEM contain-

ing 3% calf serum and antibiotics at the concentrations given above; the calf serum concentration was lowered to decrease interference from fibroblast-scaffold interactions. The isolated cells were identified as fibroblasts as described in an earlier study by the current authors.¹⁷ Both fibroblasts and embryonic nerve cells were used in 2 experiments for cell entrapment and for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays. Each experimental group consisted of 7 wells with the cells of different animals.

Isolation and culture of embryonic nerve tissue

Pregnant Wistar rats were euthanized and embryos at day 17 of gestation were isolated from the uterus in cold phosphate buffered saline (PBS, Gibco, Paisley, UK). After removal of the brains, the meninges and blood vessels were excised. The brain samples were incubated with 1 mL of enzymatic solution composed of collagenase (1 mg/mL), (Sigma, St. Louis, USA) and dispase (2 mg/mL), (Gibco, Paisley, UK) at room temperature (5 min) to remove the connective tissue. The brains were then incubated with 2 mL trypsin (5 min at 37°C), (Gibco, Paisley, UK). The trypsin was neutralized with 5 mL of medium, (MACS NeuroBrew-21, Miltenyi Biotec, Bergisch Gladbach, Germany) after which the sample was centrifuged at 1000 rpm.

The cells were resuspended with 100 μ L of medium (MACS NeuroBrew-21, Miltenyi Biotec, Bergisch Gladbach, Germany) and gently triturated (3–5 X). The cells were then seeded on laminin-coated 6-well plates (TPP, Trasadingen, Switzerland).

The embryonic nerve cells were cultured in MACS NeuroBrew-21 containing gentamicin (25 μ g/mL) and fungizone (2.5 μ g/mL). The phenotype of the cells was determined by flow cytometry. The entrapment study and MTT experiments were performed on cultures of cells derived from 9 animals.

Flow cytometry experiments

Flow cytometry experiments were performed to determine the phenotype of the isolated embryonic nerve cells. After fixation with BD Cytofix (BD Biosciences, Franklin Lakes, USA), the embryonic nerve cells were permeabilized by incubation with BD Phosflow Perm Buffer III (BD Biosciences, Franklin Lakes, USA). The samples were then washed with PBS and centrifuged (5 min, 1000 rpm).

The permeabilized cells were stained (4–8°C for 30 min) in BD Pharmingen stain buffer (BD Biosciences, Franklin Lakes, USA), with the following antibodies:

- mouse anti-Microtubule-Associated Protein 2B (MAP2B) conjugated with Alexa Fluor 488 (BD Biosciences, Franklin Lakes, USA);
- mouse anti-Myelin Basic Protein (MBP) conjugated with PE (Abcam, Cambridge, USA);

- mouse anti-Glial Fibrillary Acidic Protein (GFAP) conjugated with PE (BD Pharmingen, San Diego, USA);
- goat anti-mouse IgG2b heavy chain isotype control conjugated with FITC (BD Pharmingen, San Diego, USA);
- mouse IgG2b isotype control conjugated with PE (BD Pharmingen, San Diego, USA);
- mouse IgG1 k isotype control conjugated with Alexa Fluor 488 (BD Pharmingen, San Diego, USA).

The stained cells (3×10^4 /samples) were analyzed using a Becton-Dickinson FACScan analytical flow cytometer (Becton Dickinson, Franklin Lakes, USA).

Preparation and characterization of ternary composite collagen-based scaffolds

The collagen/hyaluronic acid/chondroitin sulfate (Col + HA + CS) matrices were constructed as described previously, with some modifications.¹⁸ In brief, a dispersion in dilute acetic acid (pH 3.2) of type I porcine collagen (Col) (Euroimplant, Rybie, Poland), hyaluronic acid (HA) from *Streptococcus equi* with molecular weight of 1.6×10^3 kDa, (Sigma-Aldrich, St. Louis, USA) and chondroitin sulfate (CS) from shark cartilage with an average molecular weight of 20 kDa, (Sigma-Aldrich, St. Louis, USA) at a ratio of 6 : 1 : 1 was freeze-dried at –50°C to form 3D sponge-shaped porous scaffolds. For comparison, sponges from pure Col were also prepared. To improve the functional properties of the scaffolds, all the collagenous sponges were treated with a carbodiimide-based process as previously described, but with significant modifications: The sponges were cross-linked at room temperature by immersion in an 80% ethanol-water solution containing 33 mM 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC) and 6 mM N-hydroxysuccinimide (NHS).^{19,20} After the reaction, the matrices were thoroughly washed in 0.1 M Na_2HPO_4 , and then with deionized water. Finally, the cross-linked matrices were again lyophilized at –55°C. All the collagen-based scaffolds were sterilized with ethylene oxide (EtO). The multi-component porous sponges were then applied as matrices for culturing cells to form a hybrid tissue-like structure intended for cell engineering.

As noted above, the addition of hyaluronic acid to an earlier investigated scaffold (Col + CS) created a tri-copolymer (Col + CS + HA), the potential of which for embryonic nerve cell and wound fibroblast culture had not been investigated prior to the present study.⁶

The porosity of each of the matrices was characterized using a Nova NanoSEM 230 scanning electron microscope (FEI Company, Eindhoven, the Netherlands) with an Apollo 40 SDEDS X-ray microanalyser (EDAX McKee Drive Mahwah, USA). The operating parameters were as follows: A low vacuum setting of 70 Pa, an accelerating voltage of 10 kV and LVD detector selected. Cross-sections or surface sections of the sponge were obtained

by breaking the specimens after freezing them in liquid nitrogen. An image analysis application was used to determine the average diameter of the pores, with at least 200 pores assessed on each specimen at $\times 100$ and $\times 400$ magnification. Fig. 1 shows the results of the pore analysis of 2 collagen-based scaffolds.

Fig. 1. Distribution of pore diameters of collagen-based scaffolds modified with EDC and NHS: (K1) scaffold composed of collagen

Fig. 2. Distribution of pore diameters of collagen-based scaffolds modified with EDC and NHS: (K2) tri-copolymer scaffold composed of collagen, chondroitin sulfate and hyaluronic acid

Cell entrapment study

Wound fibroblasts were seeded on the scaffolds at a density of 10^4 /well of 96-well plates (TPP, Trasadingen, Switzerland) and incubated for 24 h in DMEM containing 3% fetal calf serum, gentamicin and fungizone (37°C , 5% CO_2). Embryonic nerve cells were added to a 96-well plate at a density of 10^4 cells per well. They were then incubated for 24 h in MACS NeuroBrew-21 medium (Miltenyi Biotec, Bergisch Gladbach, Germany). After being washed with PBS, the samples were incubated for 30 min (37°C , 5% CO_2) with bisbenzamide ($1\text{ }\mu\text{g/mL}$ in PBS). After incubation, the scaffolds were washed with PBS, and the stained nuclei of the fibroblasts were counted under the microscope at $\times 100$ magnification.³

MTT experiments

The experiments were performed in 96-well plates (TPP, Trasadingen, Switzerland). The cells were applied to the scaffold at a density of 10^4 cells per well. After 2 or 3 days of culture, the samples were washed 3 times with PBS, and then $50\text{ }\mu\text{L}$ of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT reagent, 5 mg/mL, Sigma, St. Louis, USA) was added to each well. The samples were then incubated for 4 h at 37°C in a humid atmosphere containing 5% CO_2 . The MTT solution was removed and $200\text{ }\mu\text{L}$ DMSO was added to each sample and incubated for 10 min. After the addition of Sorensen's buffer ($0.1\text{ M NaCl} + 0.1\text{ M C}_2\text{H}_5\text{NO}_2$; pH = 10.5), the absorbance was determined at a wavelength of 570 nm using an EL $\times$ 800UV Universal Microplate Reader (BioTek Instruments Inc., Winooski, USA). Three groups of cells were studied: control cultures (2D), cultures on Col scaffolds and cultures on Col + CS + HA scaffolds. The control cells were seeded on a laminin-coated surface.

Statistical analysis

The Kruskal-Wallis test was used for the statistical analysis. Statistical differences between more than 2 independent groups were evaluated by multiple comparisons of mean ranks. The U-Mann Whitney test was used for the evaluation of statistical differences between 2 independent groups. The statistical differences between 2 dependent groups were calculated using the t-test for 2 dependent samples (when the data distribution was normal) or the Wilcoxon test (when the data distribution was not normal). The threshold of significance was $p < 0.05$. The calculations were performed using STATISTICA 12.5 software (Dell Statistica, Tulsa, USA).

Results

The Col scaffolds were found to have a homogeneously porous structure with a pore diameter of $50\text{ }\mu\text{m}$ in 70% of the pores. For 15% of the total pore volume, the pore diameter ranged from $50\text{ }\mu\text{m}$ to $140\text{ }\mu\text{m}$. However, for the tri-copolymer (Col + HA + CS), the pore diameter ranged from 24 to $160\text{ }\mu\text{m}$ for 95% of the total pore volume of the scaffold (Figs. 1 and 2).

Fibroblasts were isolated from the granulation tissue of the wound. The phenotype of the cells was confirmed by electron microscopy as described in an earlier study.¹⁷ The embryonic nerve cells were differentiated into neurons (MAP2B positive cells, Fig. 3), oligodendrocytes (MBP positive cells, Fig. 4) and astrocytes (GFAP positive cells, data not shown).⁶

Fibroblasts were able to enter both Col and Col + CS + HA scaffolds, but the Col + CS + HA scaffolds were less populated on the upper surface. Statistically signifi-

Fig. 3. Fluorescence-activated cell sorting (FACS) profiles showing the expression of microtubule-associated protein (MAP2b) on embryonic nerve cells (lower panel) compared with the control (upper panel)

Fig. 4. FACS profiles showing the expression of myelin basic protein (MBP) on embryonic nerve cells (lower panel) compared with the control (upper panel)

cant differences were observed in the numbers of fibroblasts infiltrating the Col and Col + CS + HA scaffolds (Fig. 5). At the same time, the embryonic nerve cells were entrapped by both the Col and Col + CS + HA scaffolds, but significantly higher numbers of cells were found in the former than the latter (Fig. 6).

The metabolic activity of the cells was analyzed after 2 or 3 days of culture. On the 2nd day, lower fibroblast metabolic activity was found in the Col + CS + HA scaffolds than in the controls (a 2D cell culture on a laminin-coated surface). On the 3rd day, however, the metabolic activity of the fibroblasts in Col and Col + CS + HA scaffolds was higher than in the controls. Interestingly, while the meta-

Fig. 5. Number of fibroblasts stained with bisbenzamide entrapped by scaffolds made of collagen (Col) and of collagen with chondroitin sulfate and hyaluronic acid (Col + CS + HA). Each value expresses the mean of 6 samples $\pm$ standard deviation (SD)

Fig. 6. Number of embryonic nerve cells stained with bisbenzamide entrapped by scaffolds made of collagen (Col) and of collagen with chondroitin sulfate and hyaluronic acid (Col + CS + HA). Each value expresses the mean of 6 samples $\pm$ standard deviation (SD)

bolic activity of the fibroblasts fell from day 2 to day 3 in the control cultures, the opposite occurred in the scaffolds composed of Col + CS + HA, with fibroblast activity doubling from day 2 to day 3 (Fig. 7).

On the 2nd and 3rd days of culture, embryonic nerve cells within the Col scaffolds demonstrated decreased metabolic activity compared with the controls. In the Col scaffolds, the metabolic activity of the embryonic nerve cells was significantly higher on the 3rd day of culture than on the 2nd day. Within the Col + CS + HA scaffolds, the embryonic nerve cells demonstrated increased metabolic activity on both days 2 and 3 of culture compared to the Col scaffolds (Fig. 8). Lower metabolic activity of both fibroblasts (Fig. 7) and embryonic nerve cells (Fig. 8) was noted on day 3 of culture, compared with day 2 in the control groups.

Fig. 7. Metabolic activity of fibroblasts cultured for 2 or 3 days on a laminin-coated surface (CTR), in collagen scaffolds (Col) and in scaffolds composed of collagen, chondroitin sulfate and hyaluronic acid (Col + CS + HA). Each value expresses the mean of 8 samples $\pm$ standard deviation (SD)

Fig. 8. Metabolic activity of embryonic nerve cells cultured for 2 or 3 days on a laminin coated surface (CTR), in collagen scaffolds (Col) and in scaffolds composed of collagen chondroitin sulfate and hyaluronic acid (Col + CS + HA). Each value expresses the mean of 8 samples $\pm$ standard deviation (SD)

Discussion

Earlier studies indicated that the secondary triple helix structure of collagen present within Col scaffolds remained unchanged, thus allowing the optimal biological properties of the scaffold to be used in regenerative medicine.^{3–6} In the present study, the presence or absence of cross-links, CS and HA in the collagen sponges had a significant effect on the overall structure and distribution of the interconnected pores (Figs. 1, 2). After cross-linking collagen alone, the sponges exhibited an almost homogenous distribution of pores, with 70% of the pores having a diameter of 50 μm (Fig. 1, K1). Moreover, the results also showed that pore sizes ranging from 50 to 140 μm made up about 15% of the total pore volume. Conversely, the pore size of the ternary

composite Col-HA-CS sponges, cross-linked with EDC/NHS, was significantly lower than in the collagen sponges without HA and CS (Fig. 2, K2). The largest fraction, comprising at least 70% of the total pore volume of the sponge, was occupied by pores ranging from 24 to 90 μm in size. Only 15% of the Col + HA + CS scaffolds contained pores much larger than 90 μm , i.e. ranging between 127 and 196 μm . Wang et al. reported that the pores of tri-copolymer Col + CS + HA (9 : 1 : 1) scaffolds were uniform and widely interconnected, with a mean diameter of 109 μm .²¹ The dimensions of pores in collagenous scaffolds prepared by Yannas et al. ranged from 20 to 125 μm .²² Huang et al. observed that in a collagen and glycosaminoglycan copolymer, the pore size ranged from 20 to 140 μm .²³

The present study showed good fibroblast and embryonic nerve cell entrapment within the Col scaffolds (Figs. 5, 6). The presence of a relatively high density of cells settled within the Col material implies appropriate porosity, allowing the passage of different cell types into the scaffold. Moreover, more fibroblasts than embryonic nerve cells were entrapped in the Col scaffold. This effect is believed to be caused by the size and shape of fibroblasts, which may be a better fit for the pores. In addition, the Col + CS + HA scaffolds were about 4 times less populated than the Col scaffolds and controls. Thus, supplementation of the scaffold with chondroitin sulfate and hyaluronic acid decreases the ability of the Col scaffold to catch both fibroblasts and embryonic nerve cells. In a previous study, the numbers of embryonic nerve cells in the Col scaffolds and in a Col-chondroitin sulfate composite were found to be the same.⁶ In the present study, the addition of HA may disrupt the closed, honeycomb-like structure of the Col scaffold and cause the formation of open interconnections between pores, leading to the low populations of seeded fibroblasts and embryonic nerve cells observed in the tri-copolymer Col + CS + HA scaffolds.²⁴ Although all the applied cells were entrapped within the scaffolds, different numbers of cells were found on the superficial parts of the scaffolds.

The metabolic activity of fibroblasts and embryonic nerve cells in the control groups (Figs. 6, 7) was found to be lower on the 3rd day of culture than on the 2nd day. This effect was probably caused by the limitations of a 2D culture and intensive cell-cell interactions. Bracke et al. reported inhibition of several energy-requiring activities in confluent epithelial cells, but MTT conversion increased only in confluent cells with a dysfunctional E-cadherin/catenin complex, implying that cell metabolism may be regulated by cell-cell adhesion via the E cadherin/catenin complex.²⁵ Interestingly, this effect was not observed in 3D cultures created within the scaffolds (Figs. 7, 8). Thus, both Col and Col + CS + HA scaffolds provide favorable environments for fibroblast culture.

The results of the present study confirm that the metabolic activity of fibroblasts within the 2 types of scaffold were better on the 3rd day than the 2nd day of the culture

(Fig. 7). In addition, on the 2nd day of culture, the fibroblasts within the Col + CS + HA scaffolds demonstrated lower metabolic activity than in the controls. However, a rapid increase in metabolic activity was seen in the Col + CS + HA scaffolds on the 3rd day of culture. These findings confirm those of earlier studies showing that a collagen-containing scaffold has good properties for tissue engineering.^{2,6,7} Fibroblasts seeded in collagen-gelatin sponges have been used as a drug delivery system, as they allow up-regulation of growth factor synthesis; these sponges appeared to be biocompatible with fibroblasts.²⁶ Moreover, fibroblasts cultured within scaffolds with unidirectional collagen fibers secrete regulatory factors that modulate the wound-healing process.²⁷ The collagen present in the extracellular space may also influence endogenous collagen expression.²⁸

In the present study, the Col scaffolds did not guarantee good conditions for the culture of embryonic nerve cells: The cells seeded within the Col material displayed lower metabolic activity than in either the control cultures or the Col + CS + HA scaffolds. These results correspond with the data from the authors' previous study showing that embryonic nerve cells cultured within a scaffold composed of collagen and chondroitin sulfate demonstrate better metabolic activity than those in a Col scaffold.⁶ The authors therefore recommend the tri-copolymer for embryonic nerve cell engineering.

The authors' earlier study showed that a Col scaffold supplemented with CS provides good conditions for embryonic nerve cell culture.⁶ The addition of CS improved the metabolic activity of the cells compared with those in the Col scaffold.⁶ The present study found that cells seeded in tri-copolymer scaffolds demonstrated better metabolic activity than those in scaffolds composed of Col alone. This reaction is believed to be due to hyaluronic acid modifying the structure of collagen, allowing the formation of interconnections between pores, which supports the migration of cells to internal pores, allows for better communication between cells and improves the nutrition of the seeded cells.²⁴ Since high concentrations of hyaluronic acid, chondroitin sulfate and heparin sulfate are known to be present in the brain, the collagenous scaffold was supplemented with glycosaminoglycans for nerve cell culture.²⁹ Chondroitin sulfate is known to exert a regulatory influence on the differentiation and migration of endogenous neural precursor cells.²³ In addition, stem cells seeded in scaffolds containing hyaluronic acid were characterized with better survival and differentiation.³⁰

Conclusions

The study showed that fibroblasts and embryonic nerve cells were entrapped by both of the scaffolds used (Col and Col + CS + HA). However, cell entrapment was about 4 times more efficient within the Col scaffolds than the

Col + CS + HA scaffolds for both of the tested cell cultures. Both the Col and Col + CS + HA scaffolds provided a good environment for fibroblasts; but since the scaffolds composed of Col + CS + HA guaranteed better conditions for the culture of embryonic nerve cells than Col alone, the tri-copolymer Col + CS + HA is recommended for the engineering of embryonic nerve cells.

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