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Journal of Orthopaedic Research 23 (2005) 1184–1190

Journal of Orthopaedic Research

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Growth factors and fibrochondrocytes in scaffolds

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Accepted 28 January 2005

Abstract

Four growth factors, transforming growth factor-β1 (TGF-β1), platelet-derived growth factor-AB (PDGF-AB), insulin-like growth factor I (IGF-I), and basic fibroblastic growth factor (bFGF), were tested at different concentrations for their effects on extracellular matrix (ECM) production in three-dimensional cultures of meniscal fibrochondrocytes. Cells from New Zealand white rabbits were seeded on poly-glycolic acid (PGA) scaffolds and were stimulated with growth factors for three weeks. ^{3}H-proline and ^{35}S-sulfate labels were used to measure uptake of collagen and glycosaminoglycan (GAG) components, respectively. Biochemical assays were performed to measure the total collagen, GAG, and DNA present in the scaffolds at the end of the study. TGF-β1 (10 and 100 ng/ml) stimulated both ^{3}H-proline and ^{35}S-sulfate uptake, showing a dose-dependent response for both and a temporal response for ^{35}S-sulfate uptake. IGF-I (5 ng/ml) and bFGF (25 and 100 ng/ml) showed increases in ^{3}H-proline uptake by the third week of growth factor addition. PDGF-AB did not show notable increases in uptake. Because TGF-β1 (10 and 100 ng/ml) had visibly denser scaffolds, as evidenced by gross microscopy, at 100×, and the strongest uptake responses to both ^{35}S-sulfate and ^{3}H-proline, it appears to be the most effective growth factor for use in scaffold-based approaches to tissue engineer the knee meniscus.

Keywords: Meniscus; Fibrochondrocytes; Growth factors; Collagen; Glycosaminoglycans

Introduction

Positioned between the articulating surfaces of the tibia and the femur, the knee meniscus is a fibrocartilaginous tissue that is indispensable to the function of the knee. The meniscus provides several basic functions: weight distribution [2,7,11,16,17,19,22,26], friction reduction [3,12,28], joint stability [26], and shock absorption [3,13,28]. Without the meniscus, the articular cartilage on the ends of the bones would become susceptible to damage which can lead to osteoarthritis. Once torn, the meniscus is unable to fully repair itself in the inner region due to its lack of vascularity and low cellularity [15,21]. Although it has been common practice to remove all or some of a damaged meniscus, this leaves the underlying cartilage exposed and therefore alternative procedures are being sought. One such promising solution is that of tissue engineering which can involve replacing the entire meniscus with a new piece of fibrocartilage that has been generated from a patient's own cells. This method involves seeding cells on a biocompatible scaffold, stimulating the cells with growth factors, and enhancing physical properties of the construct using mechanical stimulation [23]. Although growth factors have been studied on monolayer cultures of fibrochondrocytes, meniscal explants, in alginate beads, as well as open defects and wounds [6,8,14,23,24,27], there are no studies that look at the effects of growth factors on fibrochondrocytes seeded in scaffolds.

Growth factors have been previously shown to increase extracellular matrix (ECM) production, migration, and proliferation of meniscal cells [6,8,14,27]. Different growth factors at different concentrations can have varying effects on the type of stimulation they

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0736-0266/$ - see front matter
doi:10.1016/j.orthres.2005.01.019

provide. Imler et al. [14] looked at four different growth factors: transforming growth factor-β1 (TGF-β1) in doses of 0.05–5 ng/ml, platelet-derived growth factor-AB (PDGF-AB) in doses of 3–300 ng/ml, insulin-like growth factor I (IGF-I) in doses of 30–1000 ng/ml, and basic fibroblastic growth factor (bFGF) in doses of 1–300 ng/ml. The growth factors were applied to meniscal explants. The investigators noted that each growth factor increased the production of glycosaminoglycans (GAGs) more than they stimulated the production of collagen, and each to a different extent. The range of concentrations tested for TGF-β1 did not show saturation for proline nor sulfate incorporation. In explants cultured for 14 days, TGF-β1 showed the greatest amount of sulfate incorporation, indicating it was producing the most GAG. TGF-β1 has also been shown to increase proteoglycan production in explants (4 ng/ml TGF-β1), monolayers (1 ng/ml TGF-β1), and alginate beads (5 ng/ml TGF-β1) [8]. TGF-β1 has also increased proteoglycan synthesis in a dose-dependent manner in fibrochondrocytes [25]. Bhargava et al. [6] showed a dose-dependent increase in proliferation of meniscal fibrochondrocytes caused by PDGF-AB, hepatocyte growth factor/scatter factor, and bone morphogenetic protein-2.

The temporomandibular joint (TMJ) disc is similar to the meniscus in that it is fibrous cartilage. Detamore et al. [10] studied PDGF-AB, bFGF, and IGF-I at low (10 ng/ml) and high (100 ng/ml) concentration levels. bFGF stimulated the most proliferation and GAG synthesis, and also increased collagen production by TMJ cells. IGF-I showed the highest collagen production by the TMJ cells.

Beside choosing a growth factor, a proper biocompatible scaffolding material must be selected for three-dimensional culturing of cells. Cells need a surface on which they can attach and a framework that will encourage proper tissue formation. One advantage of using a synthetic scaffolding material over a natural material is the ability to alter the material's properties (degradation time, porosity) [9]. Poly-glycolic acid (PGA) is a polymer that promotes cell adhesion, degrades into glycolic acid (a naturally-occurring biological product), and is frequently used in orthopaedic applications [4,5]. After cells are seeded on a scaffold, they will hopefully begin to produce ECM. The growing tissue should compensate for the degradation of polymer, resulting in a completely cell-formed tissue. By using a non-woven mesh of PGA, a porous network of fibers allows attachment and migration of cells to the interior of the scaffold. The porosity also allows diffusion of media throughout the scaffold. Scaffolds can be cut into desired shapes and sizes to mimic the natural tissue.

Based on current growth factor literature, the following growth factors and concentrations were chosen for this study: TGF-β1 (1, 10, 100 ng/ml), IGF-I (5, 12.5, 50 ng/ml), PDGF-AB (10, 25, 100 ng/ml), and bFGF (10, 25, 100 ng/ml). Each growth factor was tested at a low, medium, and high concentration level to examine any saturation effects. These various growth factors have been shown to have effects on the synthetic output of meniscal fibrochondrocytes, yet they have not been applied to PGA scaffolds. It is hoped that at least one growth factor will prove successful in stimulating ECM production under these conditions.

Materials and methods

Cell harvest and culture

Fibrochondrocytes were harvested from the medial menisci of six New Zealand white rabbits. The rabbits were healthy and ranged in weight from 3.74–3.96 kg. Ketamine/xylazine was used to anesthetize and beuthanasia was used to euthanize the rabbits. Harvesting of menisci was performed under aseptic conditions. After removal, menisci were manually cut into smaller pieces using a scalpel. The tissue was then digested in 0.3% collagenase for 48 h until most large pieces of tissue had been degraded. Cells were immediately seeded in T-285 flasks with a combination Ham's F12/DMEM media (50 μg/ml ascorbic acid, 10% fetal bovine serum, 1% penicillin/streptomycin/fungizone, 1% non-essential amino acids) and stored in sterile, 5% CO_2, 37 °C incubators. All media were changed fully every three days. After 100% confluence, cells were passaged with trypsin/EDTA. A total of three passages were performed.

Seeding

PGA (Albany International, intrinsic viscosity: 0.8 dl/g, porosity: 95%) scaffolds were first sterilized using ethylene oxide. Scaffolds were then pre-wetted with ethyl alcohol, rinsed with phosphate-buffered saline (PBS), and then soaked in media overnight. Scaffolds were 3 mm in diameter and approximately 1.5 mm in height. Third passage cells were seeded onto the scaffolds at an approximate seeding density of 25 million cells/ml scaffold. Scaffolds were placed in 48-well tissue culture treated plates which had been coated with poly HEME to prevent attachment of cells to the plate itself. A total volume of 0.5 ml media (F12/DMEM) was added to each well and plates were placed on an orbital shaker in a 37 °C incubator for three days. The orbital shaker facilitated the initial attachment of the cells to the scaffolds. Plates were rotated so that optimal media flow was achieved without noticeable scaffold movement within the well. Scaffolds were subsequently left in static culture for nine days and half the media was changed every other day. The static culture period allowed the cells to stabilize and attach firmly to the scaffolds.

Growth factor addition

After the static culture period, addition of growth factors began. Every other day, half the volume of media was exchanged for a new dose of growth factor-supplemented media. Growth factors used were TGF-β1, PDGF-AB, IGF-I, and bFGF. Each growth factor was tested at a low, medium, and high concentration level: TGF-β1 at 1, 10, and 100 ng/ml; IGF-I at 5, 12.5, and 50 ng/ml; and PDGF-AB and bFGF at 10, 25, and 100 ng/ml. Three samples of each group were prepared for radiolabeling assays and six samples of each were prepared for biochemical tests. Cells seeded on scaffolds cultured in media without growth factors served as the control.

Radiolabeling assay

Once a week, for three weeks (with week 1 being one week after the start of growth factor addition) a radiolabeling assay

was performed. ^{35}S-sulfate was used to indicate the amount of sulfate being taken up by the cells for eventual GAG production. ^{3}H-proline was used to measure how much proline the cells were consuming as an indicator of collagen production. These labeled components were chosen as representative of GAG and collagen production since the cells use large amounts of these molecules when making GAG and collagen in comparison to other molecules the cells make. The labels were applied at concentrations of 10 μCi/ml, except at week 1 where only 5 μCi/ml of ^{3}H-proline was used (this was a calculation error, but is believed to not adversely have affected results since 10 μCi/ml is above the saturation limit of what the cells can take up in a given period of time). The incubation time for the cells with the label was approximately 12 h, although it was several hours longer for some groups due to constraints of processing time (the difference was held constant from week to week). To analyze each scaffold, media were removed and the scaffolds were rinsed twice with PBS. Scaffolds were placed in scintillation vials with 10 ml of scintillation fluid and measured for activity.

Biochemical analysis

After three weeks of culture in the growth factor-supplemented media, scaffolds were rinsed with PBS and lyophilized. Samples were digested with 125 μg/ml papain (Sigma) in 50 mM phosphate buffer (pH = 6.5) containing 2 mM *N*-acetyl cysteine (Sigma) and 2 mM EDTA (Sigma) at 65 °C overnight and stored then at −20 °C to await biochemical analysis. Actual amounts of collagen, GAG, and DNA (to indicate cell number) were then measured. After hydrolyzation with NaOH, scaffolds were assessed for total collagen content using a hydroxyproline assay. [chloramine-T and ehrlich's] GAG was measured with a dimethylmethylene blue dye association reagent kit (Biocolor) This kit is based on 1,9-dimethylmethylene blue binding. A PicoGreen assay (Molecular Probes) was used to quantify DNA in each scaffold.

Statistical analysis

For radiolabeling analysis, samples were run in triplicate and data are reported as mean ± standard error. For biochemical analysis, $n = 6$ was used as the sample size and data are reported as mean ± standard deviation. A one-factor analysis of variance (ANOVA) was used to determine significance of concentration for one growth factor compared to the control. If such a difference was detected, a post hoc test was performed to determine which concentrations of the given growth factor had an effect. All statistical tests employed a significance level of 95%.

Results

Gross appearance

Cells could be seen with a light microscope (100×) attached to the PGA fibers after seeding and as they gradually proliferated over time. Until about the third week of culture, there was not a noticeable visible difference among samples in terms of cell density. During the third week of culture, samples treated with medium and high concentrations of TGF-β1 (10 and 100 ng/ml respectively) were quite different from the other treatment groups. These scaffolds were densely packed with either cells and/or ECM (Fig. 1). Individual PGA fibers could not be distinguished as they were for the scaffolds under other treatments.

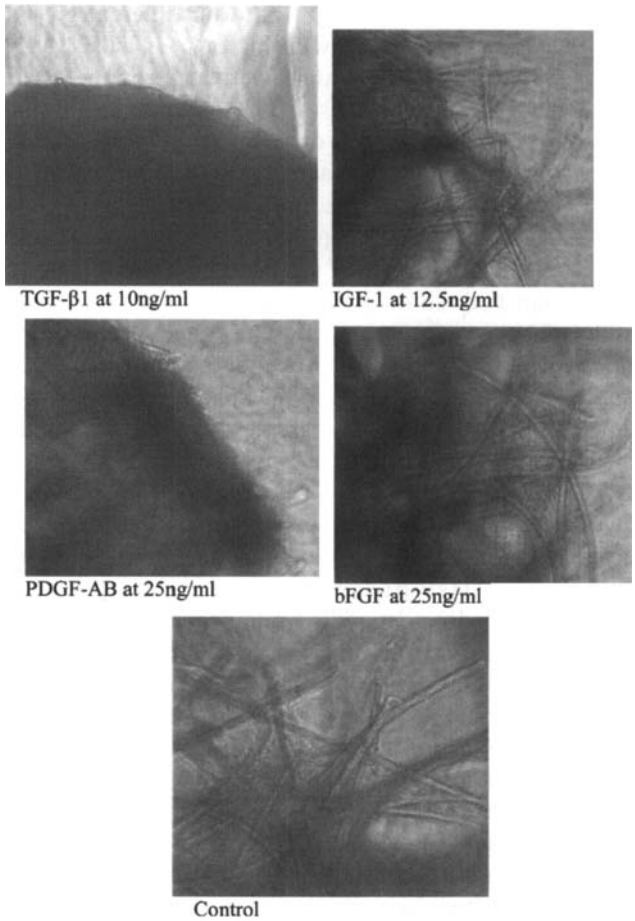

Fig. 1. The gross appearance (100×) of several scaffolds is greatly different after three weeks of growth factor addition, as evidenced by a comparison of all growth factors at medium concentration levels and the control. TGF-β1 treated scaffolds are the most densely packed and individual PGA fibers cannot be distinguished.

Radiolabeling

Collagen

Significant ^{3}H-proline uptake occurs in week three. TGF-β1 and bFGF both show a significant increase in uptake (Fig. 2). TGF-β1 shows a dose response with both the medium and high concentrations being significantly higher than the control and the low concentration, and the high having a significant increase over the medium. Compared to the control, TGF-β1 is nearly 1.8 and 2.4 times more active at its medium and high concentrations respectively. For bFGF, the medium and high concentrations both show a significant increase over the control, and the medium is significant over the low. bFGF at its medium and high concentrations is approximately 1.7 and 1.6 times as active, respectively, as the control. The high concentration of TGF-β1 is significantly higher in uptake than the high concentration of bFGF at this time point. All data for ^{3}H-proline uptake are included in Table 1(Panel a–Panel c). Neither

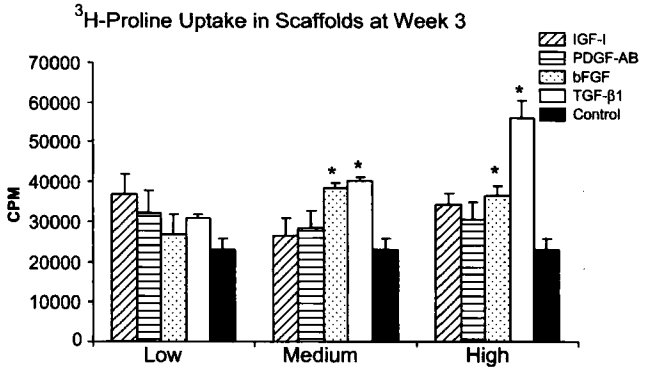

Fig. 2. After three weeks of stimulation, TGF-β1 and bFGF show significant ^{3}H-proline uptake over the control. Asterisks indicate significance over the control. $p < 0.05$.

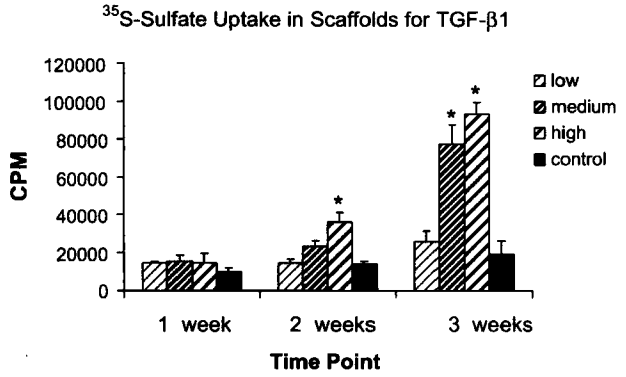

Fig. 3. TGF-β1 is the only growth factor to show significant increases over the control in ^{35}S uptake. An increase in activity is seen temporally and with dose. Asterisks indicate significance over the control. $p < 0.05$.

IGF-I nor PDGF-AB show significant increases in ^{3}H-proline uptake at any time point.

GAG

A strong increasing trend for ^{35}S-sulfate uptake is shown by TGF-β1 (Fig. 3). In week 2, the highest concentration has a significant effect in comparison to the lower levels and the control (approximately 2.6 times greater than the control), and the medium concentration is trending towards being higher than the lower concentration as well as the control. By week 3, the medium and high concentrations are both significantly higher than the low concentration and the control. The medium and high concentrations are 4 and 5 times higher than the control respectively, but are not significantly different from each other. After two weeks of growth factor addition, PDGF-AB actually shows a significant decrease in ^{35}S-sulfate uptake. Most notably, the control is nearly 1.6 times greater than the medium concentration (25 ng/ml) of PDGF-AB at this time point. The other growth factors do not show significant differences from the control or notable trends in ^{35}S-sulfate uptake (Table 1(Panel a–Panel c)).

Biochemistry

When the total amounts of collagen were measured for each scaffold and normalized by cell number, only two growth factors showed significantly more

Table 1
^{3}H-proline and ^{35}S-sulfate incorporation data for scaffolds treated with growth factors: at low concentration levels (Panel a), at medium concentration levels (Panel b) and at high concentration levels (Panel c)

Growth factor (ng/ml)	Week 1		Week 2		Week 3	
	^{3}H-proline	^{35}S-sulfate	^{3}H-proline	^{35}S- sulfate	^{3}H-proline	^{35}S-sulfate
Panel a						
TGF-β1 (1)	9888 ± 666	14,544 ± 972	24,568 ± 1068	14,631 ± 1627	30,757 ± 1078	26,000 ± 5320
IGF-I (5)	11,153 ± 1092	13,140 ± 892	28,305 ± 374	14,121 ± 2422	37,022 ± 4918	12,215 ± 1017
PDGF-AB (10)	11,364 ± 955	12,283 ± 90	23,452 ± 1717	8351 ± 736*	32,026 ± 5680	13,474 ± 1450
bFGF (10)	11,923 ± 644	13,888 ± 2105	27,884 ± 1079	17,720 ± 4289	26,933 ± 4760	14,054 ± 828
Control (0)	8690 ± 1260	9538 ± 2158	31,661 ± 5999	13,775 ± 1633	22,986 ± 2847	19,046 ± 7304
Panel b						
TGF-β1 (10)	13,797 ± 2139	15,369 ± 2972	31,574 ± 3459	23,029 ± 3041	40,393 ± 811*	77,294 ± 10,364*
IGF-I (12.5)	14,077 ± 2907	15,956 ± 1533	35,859 ± 2604	15,501 ± 1763	26,519 ± 4304	16,642 ± 1137
PDGF-AB (25)	9761 ± 503	9675 ± 933	22,942 ± 1426	8247 ± 1639*	28,354 ± 4557	18,644 ± 282
bFGF (25)	10,529 ± 1754	11,999 ± 1732	26,330 ± 3993	12,682 ± 2087	38,436 ± 1226*	19,578 ± 3032
Control (0)	8690 ± 1260	9538 ± 2158	31,661 ± 5999	13,775 ± 1633	22,986 ± 2847	19,046 ± 7304
Panel c						
TGF-β1 (100)	13,063 ± 879	14,500 ± 5020	28,305 ± 374	36,168 ± 4918*	56,099 ± 4346*	93,363 ± 6135*
IGF-I (50)	11,203 ± 930	19,752 ± 6105	24,908 ± 1632	17,993 ± 4068	34,505 ± 2564	18,643 ± 2539
PDGF-AB (100)	9542 ± 920	11,749 ± 1231	31,279 ± 4380	11,537 ± 890	30,496 ± 4455	15,823 ± 1718
bFGF (100)	11,942 ± 860	11,120 ± 747	35,624 ± 4220	21,310 ± 535	36,710 ± 2434*	17,761 ± 3070
Control (0)	8690 ± 1260	9538 ± 2158	31,661 ± 5999	13,775 ± 1633	22,986 ± 2847	19,046 ± 7304

All results are mean ± standard error. All units are cpm Asterisks indicate a significant difference compared to the control ($p < 0.05$).

Table 2
Biochemical data for scaffolds

Concentration (ng/ml)	GAG (μg/10^4 cells)	Collagen (μg/10^4 cells)	Cells/scaffold (/10^4)
PDGF-AB			
10	0.20 ± 0.13	0.20 ± 0.17	15.86 ± 2.12
25	0.23 ± 0.14	0.12 ± 0.05	19.69 ± 5.31
100	0.11 ± 0.11	0.25 ± 0.33	20.64 ± 2.59
TGF-β1			
1	0.20 ± 0.25	0.10 ± 0.12	19.79 ± 2.19
10	0.39 ± 0.23	0.17 ± 0.13	15.06 ± 8.56
100	0.09 ± 0.20	0.21 ± 0.31	13.38 ± 5.52
IGF-I			
5	0.22 ± 0.10	0.42 ± 0.12*	26.24 ± 2.24*
12.5	0.22 ± 0.05	0.21 ± 0.19	24.59 ± 2.26*
50	0.22 ± 0.02	0.06 ± 0.07	23.23 ± 1.07*
bFGF			
10	0.25 ± 0.10	0.14 ± 0.13	26.05 ± 2.93*
25	0.15 ± 0.19	0.16 ± 0.10	26.42 ± 1.02*
100	0.31 ± 0.07	0.28 ± 0.13*	25.19 ± 2.00*

Control data: GAG = 0.13 ± 0.16 μg/10,000 cells; collagen = 0.05 ± 0.07 μg/10,000 cells; cell/scaffold(/10,000)=16.39 ± 0.94

All data are mean ± std dev. Asterisks indicate significance compared to control ($p < 0.05$).

production than the control: IGF-I and bFGF (Table 2). It should be noted that many groups exhibited many fold increase in ECM components, especially collagen, but due to the large error bars most of those were not significant. The high concentration of bFGF showed approximately a 5.6-fold increase over the control while the low concentration of IGF-I showed approximately an 8.4-fold increase over the control. All concentrations of IGF-I and bFGF showed an increase in cell number over the control. Once normalized by cell number, none of the scaffolds contained significantly more GAG than the control.

Discussion

The dry weight of the meniscus is 60–70% collagen (mostly types I and II) and 5% GAGs [12,18]. The optimal growth factor for use in tissue engineering the meniscus will be one that can increase the production of these ECM components, as compared to a control, when the cells are seeded on scaffolds. The increased ECM is needed to lend the engineered construct proper mechanical functionality. The knee meniscus must withstand 45–75% of the load applied to the joint [1,2,22]. The exact load depends on the species under consideration, the extent of flexion or extension in the joint, and the health of the joint. Collagen provides the tissue with ability to resist tension and GAGs provide the tissue with compressive resistance. As the meniscus is compressed, it distends outward, while remaining anchored at its ends, creating a hoop stress circumferentially. Since the meniscus is exposed to both tension and compression, it is important to promote both collagen and GAG production in tissue-engineered constructs of the meniscus. The focus of this study was aimed at the interaction of four growth factors at three concentration levels each and fibrochondrocytes that are seeded on PGA scaffolds, in an effort to identify how the growth factors affect ECM production.

By seeding meniscal fibrochondrocytes on PGA scaffolds and applying different growth factors at different concentrations, this study was able to select optimal biochemical conditions for tissue engineering the knee meniscus. The ideal growth factor and concentration would stimulate the cells to upregulate both collagen and GAG production when compared to a control (no growth factors). The production of these components was evaluated by gross appearance, histology, biochemical analysis, and radiolabeling.

Scaffolds treated with TGF-β1 (medium and high concentrations) visually appeared to have more cells and/or ECM coating their fibers than scaffolds treated with other growth factors. Results from the radiolabeling assay support this observation in that TGF-β1, at medium and high concentrations, increased the uptake of both collagen (^{3}H-proline) and GAG (^{35}S-sulfate) components more than the control as well as the other growth factors. The gross appearance of the scaffolds concurs with our previous investigation of the same growth factors and concentrations on monolayer cultures: TGF-β1 (10 and 100 ng/ml) produced monolayer cultures that had densely packed cells and appeared to have a sheet of matrix covering them [20]. It is evident that these scaffolds, treated with TGF-β1, have substantially more matrix production or cell proliferation than the other treatment groups.

After three weeks of growth factor addition, differences in ^{3}H-proline uptake in comparison to the control were quantified. At week 3, TGF-β1 and bFGF showed higher ^{3}H-proline uptake than the control at various concentrations. It is TGF-β1 that shows the highest increase at its high concentration level. This correlates well with the visual observations that indicate considerably more matrix in these scaffolds. TGF-β1 at 10 and 100 ng/ml is the best inducer of ^{3}H-proline uptake and consequently, collagen production. Since the high concentration (100 ng/ml) is 1.4 times greater in uptake over the medium concentration (10 ng/ml), saturation has not been achieved for ^{3}H-proline uptake. As this growth factor is quite expensive to purchase, financial considerations become important for using even higher doses of TGF-β1. The concentrations of bFGF used do show a saturation effect in terms of ^{3}H-proline uptake.

The biochemical data for collagen are inconclusive. The only significant results were for collagen production by IGF-I (5 ng/ml) and bFGF (100 ng/ml). Most of the raw data from biochemical analysis are at the lower limit of, and generally below, the standards used. As a result, the assays yield large variation for most samples. It is possible some of the collagen produced was washed out of the scaffolds. However, since the culture time of this study was relatively short and there was no mechanical stimulation, perhaps not enough collagen was produced to be accurately measured by the hydroxyproline assay. Due to the limitations of the assay, and the large amount of variation seen within sample groups, only radiolabeling data are weighted as a factor in the determination of growth factor effects on collagen production. Radiolabeling is more sensitive and in this study, a more accurate indicator of growth factor effectiveness.

TGF-β1 shows a consistent temporal and dose response in terms of ^{35}S-sulfate uptake. It is also the only growth factor to show any significance over the control in terms of ^{35}S-sulfate uptake. The medium and high concentrations increase the cell-seeded scaffolds' effectiveness in ^{35}S-sulfate uptake as a function of time, while the low concentration is not higher than the control. After three weeks of growth factor addition, the medium and high concentrations of TGF-β1 are not significantly different from each other. This indicates a plateau effect of concentration over time. These results for TGF-β1 seem to agree with data from meniscus explants: TGF-β1 stimulated the most sulfate uptake and did not reach a saturation effect with 5 ng/ml used as the high concentration [14]. As with the collagen biochemical assays, the biochemical assays to detect GAG fell below or nearly below the detectable limit of the standards. This lack of substantial ECM content further supports the idea that longer culture times and biomechanical stimulation are needed. However, the radiolabeling assay is sensitive enough to detect molecular uptake and can provide a valid assessment of which growth factor(s) most stimulates a cell to upregulate ECM production.

Because of the higher content of DNA in all scaffolds treated with IGF-I and bFGF, these growth factors seem to have a proliferative effect on meniscal fibrochondrocytes. This finding may indicate that increases in uptake shown in these treatment groups may be due to an increase in cell number. As in the native meniscus, cell number in tissue-engineered constructs is limited by diffusion. It is therefore more advantageous to use a growth factor that increases ECM production per cell, as is indicated by the increased uptake of ^{3}H-proline and ^{35}S-sulfate induced by TGF-β1 which is not correlated with an increase in cell number.

TGF-β1 shows the strongest uptake response for both collagen and GAG components. This response is dose dependent and, for ^{35}S-sulfate uptake, increases temporally relative to the control. Since GAG and collagen are both important in the meniscus, TGF-β1 is the recommended growth factor for subsequent use in scaffold engineering for the knee meniscus. Cells stimulated by TGF-β1 are activated to drastically increase their uptake of ECM components, indicating an increase in ECM production. As far as the optimal concentration of TGF-β1 to be used, either 10 or 100 ng/ml appear to be valid choices. There seems to be a plateau effect in terms of ^{35}S-sulfate uptake over time, with these two concentrations not being significantly different from each other in uptake at week 3. There is about a 100% increase in the amount of ^{3}H-proline uptake at week 3 using the high concentration over the medium. However, there is not a consistent significant difference between the medium and high concentrations to warrant using one over the other. Considering its additional economic benefits, it is therefore suggested that a concentration of 10 ng/ml of TGF-β1 be used for scaffold-based tissue engineering of the meniscus.

Acknowledgement

This work was partially supported by a grant from The National Institute of Arthritis and Musculoskeletal and Skin Diseases (Grant # R01 AR47839-2).

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