

METHODS AND COMPOSITIONS FOR INHIBITION OF CELL INVASION AND FIBROSIS USING DEXTRAN SULFATE

1. FIELD OF THE INVENTION

The present invention is directed to compositions comprising biocompatible anionic polymers and methods using such compositions to inhibit fibrosis, and attendant complications such as scar formation and surgical adhesions. Compositions and methods to inhibit glial cell invasion, neurite outgrowth and bone growth are also provided.

2. BACKGROUND OF THE INVENTION

Surgical adhesions—attachment of organs or tissues to each other through scar tissue—can produce clinical problems. The formation of scar tissue is a normal sequel to surgery or other tissue injury and is required for proper wound healing. In some cases, however, the scar tissue overgrows the intended region and creates surgical adhesions. These scar tissue surgical adhesions restrict the normal mobility and function of affected body parts. Where peripheral nerves are involved, fibrous adhesions can elicit severe pain during normal movement. Furthermore scars and keloid tissue (raised scar tissue) are often unsightly and present psychological and emotional problems.

2.1. Peridural Fibrosis

A clinically important example of detrimental scar formation occurs with peridural fibrosis. This condition leads to recurrent low back pain after lumbar laminectomy and diskectomy (Cauchoix et al., 1978, *Spine* 3:256–259; Jackson, 1971, *J. Bone Joint Surg.* 53B:409–616; Pheasant, 1985, *Orthop. Clin. North Am.* 6:319–329; Yong-Hing et al., 1980, *Spine* 5:59–64). Tissue scar formation restricts nerve root mobility and has been correlated with recurrent radicular pain, often in the same distribution as the previously herniated disk (Benoist, M. et al., 1980, *Spine* 5:432–436).

2.2. Prevention of Detrimental Scarring

A number of workers have studied the effectiveness of various treatments for preventing peridural fibrosis. Fat grafts have been used with some success to prevent or ameliorate scar formation (LaRocca and Macnab, 1974, *J. Bone Joint Surg.* 56B:545–550; Langensköld and Kivilvoto, 1976, *Clin. Orthop.* 115:82–85; Gill et al., 1985, *Spine* 10:662–667; Gill et al., 1979, *Spine* 4:176–185; Yong-Hing et al., 1980, *Spine* 5:59–64). Gelfoam (denatured collagen gel) and silastic membranes showed some effectiveness in preventing adhesions (LaRocca and Macnab, supra); later studies, however, indicated that gelfoam was ineffective or promoted scar formation (Gill, 1985 supra; Gill, 1979, supra; Yong-Hing, supra). Songer et al. reported that sodium hyaluronate, but not gelfoam or anterior fat grafts, retarded fibrosis and reduced fibroblast invasion in a dog model (1990, *Spine* 15:550–554).

2.3. Cell Invasion and Attachment

Previous work by Snow et al., (1990, *Exp. Neurol.* 309: 111–130) demonstrated that keratan sulfate/chondroitin sulfate-proteoglycan (KS/CS-PG) is inhibitory to neurite outgrowth from embryonic (E-9) chick dorsal root ganglia (DRGs). Neurites either stopped abruptly or turned and travelled along the KS/CS-PG stripe border. This phenomenon was dependent upon the concentration of the pro-

teoglycan, with intermediate concentrations producing intermittent patterns of crossing.

A number of studies have considered the role of proteoglycans in cell attachment. Unfractionated cartilage proteoglycans, and to a lesser extent a purified cartilage component, chondroitin sulfate, were found to inhibit fibroblast binding to collagen and fibronectin in vitro (Rich, et al., 1981, *Nature* 293:224–226). Dermatan sulfate proteoglycan (DS-PG) was observed to inhibit the attachment and spreading of 3T3 fibroblasts on plasma fibronectin-coated culture substrata (Lewandowska et al., 1987, *J. Cell Biol.* 105:1443–1454; Rosenberg, L. C. et al., 1986, *CIBA Foundation Symposium* 124:47–68). Dextran sulfate and high molecular weight heparin decreased the initial rate of attachment of chinese hamster ovary and G-8 mouse myoblast cells to collagen (Klebe, R. J. and P. J. Mock, 1982, *J. Cell. Physiol.* 112:5–9). Proteoglycan isolated from cartilage, freed from glycoproteins and hyaluronic acid, retards attachment of a variety of cell types, including chick embryo fibroblasts, to tissue culture plastic and collagen (Knox, P. and P. Wells, 1979, *J. Cell Sci.* 40:77–88). However, the glycosaminoglycans keratan sulfate, chondroitin sulfate and hyaluronic acid showed no inhibition of cell attachment (Knox and Wells, supra).

Glycosaminoglycans (GAGs), principally heparan sulfate and dermatan sulfate, also have been identified as mediators of fibroblast (murine 3T3 cell) attachment to fibronectin (Laterra, et al., 1980, *Proc. Natl. Acad. Sci. U.S.A.* 77:6662–6666). The presence of fibronectin or hyaluronic acid, or both, in a 3-dimensional type I collagen sponge was found to enhance wound healing in vivo, and to support fibroblast invasion with resulting collagen deposition in vitro (Doillon, C. J. et al., 1987, *Biomaterials* 8:195–200).

Two glial, two epithelial and one fibroblastic cell line showed comparable or decreased binding to collagen-glycosaminoglycan relative to collagen (Reichard-Brown and Akeson, supra). Hyaluronic acid inhibits aggregation of 3T3 fibroblasts (Underhill, C. and Dorfman, A., 1978, *Exp. Cell Res.* 117:155–164), and chondroitin sulfate appears to prevent adhesion of leukocytes to endothelium (Fibbi, G. et al., 1983, *Biochem. Biophys. Acta* 762:512–518).

Studies of the composition of substratum adhesion sites of fibroblasts indicate that cell-surface proteoglycans, predominantly heparan sulfate proteoglycan, play an important role in close and focal contact adhesions (Culp, L. A. et al., 1986, *CIBA Foundation Symposium* 124:158–83; Izzard, C. S. et al., 1986, *Exp. Cell Res.* 165:320–336; Lark, M. W. et al., 1985, *Fed. Proc.* 44:394–403; Rollins, B. J. and L. A. Culp, 1979, *Biochem.* 18:141–148; Culp, L. A. et al., 1979, *J. Supramol. Struct.* 11:401–427; Culp, L. A. et al., 1978, *J. Cell Biol.* 79:788–801; Culp, L. A. and H. Bensusan, 1978, *Nature* 273:680–682; Cornic, M. et al., 1980, *Eur. J. Cell Biol.* 22:262). Secreted glycosaminoglycans in the substrate-attached material, rather than fibronectin and collagen, appear to play a rate limiting role in the adhesion process of a skeletal mouse myoblast line (Schubert, D. and M. La Corbiere, 1980, *J. Biol. Chem.* 255:11564–569). A proteoglycan secreted by rat yolk-sac tumor cells inhibited tumor cell binding to fibronectin and type I collagen, but not type IV collagen, which bound 12 times less proteoglycan than did type I collagen (Brennan, M. J. et al., 1983, *Cancer Res.* 43:4302–4307).

2.4. Adhesive Proteins

The bioadhesive proteins of mussels, oysters and barnacles adhere to a variety of surfaces underwater with high