

透明質酸的製備與應用

劉湘蘭、顏裕鴻

E-mail: 9608237@mail.dyu.edu.tw

摘要

透明質酸 (hyaluronic acid) 又稱為玻尿酸，是由D - 葡萄糖醛酸與N - 乙酰基葡萄糖胺以雙糖單元重複鍵結所組成之直鏈高分子多醣體，平均分子量介於十萬到一千萬Dalton之間。透明質酸通常由動物軟組織以有機溶劑萃取方法獲得，這種來源萃取方法的缺點為品質及來源較不穩定。傳統上透明質酸大多從雞冠萃取而獲得。由於以動物組織來源萃取過程較複製，生產成本高，近年來已將注意力逐漸發展至由微生物醱酵生產透明質酸。透明質酸在生物體內，扮演著許多的角色。普遍存在於細胞外間質以及生物體液之中，它能提供細胞的支持作用，並能調控細胞間的附著，細胞的聚集、移動、增殖與分化等作用。透明質酸水溶液具有特殊的黏彈性質，在動物體中可以提供許多的生理功能，如保護、潤滑、支持等功能。由於透明質酸具有特殊生物相容性、獨特的流變學特性和保濕能力等特點，因此在生物醫學、保健食品、化妝品工業等相關領域上有廣泛的應用。

關鍵詞：透明質酸(玻尿酸)；生物相容性；製備；應用

目錄

目錄 封面內頁 簽名頁 授權書.....	iii	中文摘要.....	iv	英文摘要.....	v
誌謝.....	vi	目錄.....	vii	圖目錄.....	xi
表目					
錄.....	xii	第一章 前言.....	1	第二章 透明質酸的介紹.....	3
第一節 透明質酸的發現.....	3	第二節 透明質酸的結構.....	3	一、一級結構 (螺旋結構).....	3
二、二級結構 (網狀結構).....	4	三、三級結構.....	5	第三節 透明質酸的物化性質.....	9
一、透明質酸的降解反應.....	9	二、透明質酸的分子量.....	10	三、透明質酸的黏彈性質.....	11
四、透明質酸的異化作用.....	13	五、透明質酸納鹽在水溶液之熱穩定性.....	15	六、透明質酸對組織內滲透壓的影響.....	15
七、透明質酸的排除效應.....	17	第四節 透明質酸的生理功能.....	17	一、透明質酸的保濕能力.....	17
二、透明質酸的緩衝作用.....	18	三、透明質酸對細胞的作用.....	18	四、受體結合作用.....	21
五、組成多種間質.....	23	第五節 透明質酸的生化合成.....	23	一、透明質酸合成機制.....	23
二、透明質酸合成的調節作用.....	26	第三章 透明質酸的製備.....	29	第一節 動物組織體萃取.....	30
一、動物來源萃取.....	30	二、不同動物組織生產製備法.....	33	三、Balazs純化製備法.....	35
四、透明質酸蛋白質修飾法.....	36	第二節 生化工程技術萃取.....	37	一、發酵簡介.....	37
二、發酵工程.....	38	三、微生物發酵透明質酸.....	39	四、生產菌種來源.....	40
五、優良菌種選育.....	41	六、透明質酸發酵條件.....	45	七、發酵液純化法.....	47
第三節 化學合成法.....	49	一、高分子結構.....	49	二、化學合成.....	49
第四節 透明質酸的檢測.....	53	一、葡萄糖醛酸測定法.....	53	二、葡萄糖胺測定法.....	54
第四章 透明質酸的應用.....	55	第一節 透明質酸於保健食品方面的應用.....	55	一、透明質酸保健食品的使用來源.....	55
二、透明質酸保健食品的功能.....	56	三、透明質酸的吸收與代謝.....	57	四、透明質酸保健食品的劑型種類.....	58
五、透明質酸保健食品的展望.....	59	第二節 透明質酸於化妝品方面的應用.....	59	一、透明質酸化妝品的使用來源.....	59
二、皮膚組織中透明質酸的分布.....	60	三、透明質酸在皮膚組織的生理功能.....	61	四、透明質酸在化妝品的應用功能.....	63
第三節 透明質酸於醫學方面的應用.....	68	一、黏性補充物質的應用.....	68	二、整型手術的應用.....	69
三、眼科醫學方面的應用.....	70	四、創傷癒合方面的應用.....	72	五、手術後組織抗沾黏的應用.....	73
六、藥物釋放上的應用.....	74	七、輔助診斷疾病應用.....	75	第四節 透明質酸衍生生物的應用.....	76
一、交聯透明質酸.....	77	二、透明質酸酯化.....	79	第五章 結論.....	81
參考文獻.....	83				

參考文獻

1. 三谷、?田薰、須貝哲郎。1988。玻尿酸之分子量的保濕能。香妝會志。12:50-59。
2. 山本肇。1998。????酸?美容食品???用。Fragrance J. 26 (7) :70-75。
3. 王雲萍。1994。生物科技的寵兒 玻尿酸。化工資訊。5:44-49。
4. 尹蓮芳，劉璐。2001。慢性腎衰

患者血透明質酸、層黏連蛋白、Ⅰ型膠原、Ⅱ型膠原與腎功能的關係。Jiangsu Medical Journal. 27 (1):42-43. 5.田蔚城 編著。2001。生物技術的發展與應用。第3-4頁。九州圖書文物有限公司。台北，臺灣。6.江晃榮。2004。生物醫學的寵兒—生體高分子膠原蛋白與玻尿酸。工資訊與商情。17:73-79。7.池木根，劉昌玲。1995。豬皮透明質酸的製備和分析。軍事醫學科學院院刊。19 (1):55。8.朱廣華，方煜平。1996。透明質酸製備方法及應用研究的進展。中國醫藥工業雜誌。27 (8):382-385。9.李榆梅主編。2001。全國普通醫藥中等教材微生物學。中國醫藥科技出版社。第3版。北京。10.李寧遠。2005。膠原蛋白與玻尿酸。健康世界。240 (360):21-22。11.杜平中。1998。透明質酸的皮膚保健功能。中國生化藥物雜誌。19 (5):283-284。12.沈渤江，張天民。1986。公雞冠透明質酸的製備及其理化性質。醫藥工業。17 (7):291-294。13.和地陽二。1990。???????? - 有用物?????展望。????????-??。2:21-29。14.花崗一雄，韓晶岩。1990。疼痛的生理和傳導路。日本醫學介紹。11 (4):145-146。15.胡帽穎，顧文卿。2003。透明質酸交聯酯化衍生物的製備及醫學應用進展。透析與人工器官。14 (3):30-46。16.凌沛學，張天民。1987。牛眼玻璃體透明質酸的研究及應用。醫藥工業。18 (7):295。17.凌沛學，賀豔麗，白若琬。2000。玻璃酸鈉結構及理化性質的研究進展。中國生化藥物雜誌。21 (3):152-154。18.高玉瓊，劉建華。1996。不同組織來源透明質酸的分離純化及其鑑定。生物化學雜誌。12 (2):215-218。19.高鋒，孔憲濤。1994。腫瘤患者血清透明質酸，層黏蛋白檢測的臨床意義。腫瘤。14 (1):9-11。20.高山健一郎。1988。????酸 - ??生產?應用。化學?生物。26:308-315。21.常育，王啟祥，蘭英，黨煜。2005。透明質酸的製備方法。美中國際創傷雜誌。4 (2):60-61。22.許志強，劉平。1999。細胞外基質的結構與功能。肝臟。4 (2):92-93。23.郭學平，凌沛學。2000。透明質酸的生產。藥物生物技。7 (1):61-64。24.郭學平。1994。發酵法製備透明質酸。日用化學工業。2:47-48。25.黃定國。2001。透明質酸之開發與應用。菌種保存及研究簡訊。14 (3):1-9。26.張文會。1997。皮膚的乾燥與保濕。日用化學品科學。93 (2):9-12。27.趙景聯。1991。透明質酸的製備技術。適用技術與發展。4:22-23。28.董科云，王海英，郭學平，樂貽宏。2006。透明質酸鈉熱穩定性的研究。食品與藥物。8 (1):29-33。29.楊春濤。1998。透明質酸乾粉及其製劑的穩定性觀察。中國生化藥物雜誌。19:273-274。30.楊好，熊文說，應國清，易喻，王鴻。2006。交聯透明質酸衍生物的製備與應用進展。化工進展。25 (12):1410-1414。31.楊美桂 編著。2003。微生物學。第228-232頁。藝軒圖書出版社。台北，臺灣。32.閻家麒，朱建梅。1992。高分子量透明質酸製備工業研究。中國醫藥工業雜誌。23 (8):337-338。33.蔡曉雯、許富銀。2004。膠原蛋白與透明質酸在生醫工程上的應用。工資訊與商情。13:64-71。34.蔣秋燕，凌沛學，?思玲，林洪，張天民。2005。口服透明質酸在大鼠體?吸收機制的研究。中國藥學雜誌。40 (23):1811-1813。35.劉玉蘭，王娟。1999。皮膚保濕劑及其性能評價方法的研究。日用化學工業。5:52-54。36.慶月。2005。透明質酸的製備和生產應用。醫藥化工。12:30-35。37.蘇志遠 編著。1993。生物技術。第13-26頁。黎明文化事業(股)公司。台北，臺灣。38.Arufo, A., Stamenkovic, L. and Mdnick, M. 1990. CD44 is the principal cell surfact receptorfor hyaluronate. Cell. 61(7): 303. 39.Balazs, E. A., Rydell, N. W. and Butler, J. 1970. Hyaluronic acid in synovial fluid. Effect of intra-articular injection of hyaluronic acid on the clinical symptoms of arthritis in track horses. Acta. Vet. Scand. 11(2): 139-155. 40.Balazs, E. A. 1979. Ultrapure hyaluronic acid and the use therefore. U.S. Patent 4141973. 41.Balazs, E. A., Leshchiner, A., Leshchiner, A., Band, P. 1987. Chemically modified hyaluronic acid preparation and method of recovery thereof from animal tissues. U.S. Patent 4713448. 42.Balaz, E. A. 1990. Medical applications of hyaluronan and its derivatives. In Cosmetic and Pharmaceutical Applications of Polymers 293. Plenum press, New York, U.S.A. 43.Balazs, E. A., Leshchiner, A., Band, P. 1986. Isolating modified hyaluronic acid. U.K. Patent 2172295. 44.Balazs, E. 1990. The physical properties of synovial fluid and the specific role of hyaluronic acid. Curr. Ther. Res. 47: 437-443. 45.Balazs, E. A. and Band, P. 1984. Hyaluronic acid:its structure and use. Cat. Inist. 99(6): 65-72. 46.Bentley, J. P. 1968. Mucopolysaccharide synthesis in healing wounds. In: Dunphy. (J.E., Van Winkle, W. Jr., eds) Repair and regeneration. p.151-160. McGraw-Hill. New York. U.S.A. 47.Betina, V. 1995. Differentiation and secondary metabolism in some prokaryotes and fungi. Folia Microbiol. 40(1): 51-67. 48.Bitter, T. and Muir, H. M. 1962. A modified uronic acid carbazole reaction. Anal. Biochem. 4: 330-334. 49.Bothner, H. and Wik, O. 1987. Rheology of hyaluronate. Acta Otolaryngol (Stockh). 442: 25-30. 50.Carbrera, R. C., Siebert, J. W., Eidelman, Y., Gold, L. I., Longaker, M. T., Garg, H. G. 1995. The in vivo effect of hyaluronan associated protein-collagen complex on wound repair. Biochem. and Molecular Biol. Int. 37(1): 151-158. 51.Chong, B. F., Nielsen, L. K. 2003. Aerobic cultivation of Streptococcus zooepidemicus and the role of NADH oxidase. Biochemical Engineering Journal. 16: 153-162. 52.Clarris, B. J. and Fraser, J. R. 1968. On the pericellular zone of some mammalian cells in vitro. Exp. Cell. Res. 49(1): 181-193. 53.Cutler, R. G. 1991. Antioxidants and aging. Am. J. Clin. Nutr. 53(1): 373-379. 54.Dillon, P. W., Keefer, K., Blackburn, J. H., Houghton, P. E. and Krummel, T. M. 1994. The extracellular matrix of fetal wound:hyaluronic acid controls lymphocytes adhesion. J. Surg. Res. 57(1): 170-173. 55.Duranti, F., Salti, G., Bovani, B., Calandra, M. and Rosati, M. L. 1998. Injectable hyaluronic acid gel for soft tissue augmentation. Dermatol. Surg. 24(12): 1317-1325. 56.Elson, L. A., Morgan, W. T. J. 1933. A colorimetric method for the determination of glucosamine and chondrosamine. Biochem. J. 27(6): 1824-1828. 57.Evered, D. and Whelan, J. 1989. The biology of hyaluronan, Ciba Foundation Symposium 143. John Wiley and Sons. 6-15. 58.Francesco, D.V., Aurelio, R., 1986. New polysaccharide esters and their salts. EP. Patent 216453. 59.Fraser, J. R., Laurent, T. C., Pertoft, H., Baxter, E. 1981. Plasma clearance, tissue distribution and metabolism of hyaluronic acid injected intravenously in the rabbit. Biochem. J. 200(2): 415-424. 60.Frebourg, T., Delpech, B., Bercoff, E., Senant, J., Bertrand, P., Deugnier, Y., Bourteille, J. 1986. Serum hyaluronate in liver diseases: study by enzymoimmunological assay. Hepatology. 6(3): 392-395. 61.Ghosh, P. 1994. The role of hyaluronic acid (hyaluronan) in health and disease: Interactions with cells, cartilage and components of synovial fluid. Clin. Exp. Rheumatol. 12(1): 75-82. 62.Gibbs, D. A., Merrill, E. W., Smith, K. A., Balazs, E. A. 1968. Rheology of hyaluronic acid. Biopolymers. 6: 777-791. 63.Hascall, V. C. and Laurent, T. C. 1997. Hyaluronan: structure and physical properties. <http://www.glycoforum.gr.jp>. 64.Hayase, S., Oda, Y., Honda, S. and Kakehi., K. 1997. High-performance capillary electrophoresis of hyaluronic acid: Determination of its amount and molecular mass. J. Chromatography A. 768: 295-305. 65.Huey, G., Stair, S., Stern, R. 1990. Hyaluronic acid determinations. Optimizing assay parameters. Matrix. 10(2): 67-74. 66.Kendall, F. E., Heidelberger, M. and

Dawson, M. H. 1937. A serologically inactive polysaccharide elaborated by mucoid strains of group A hemolytic streptococcus. *J. Biol. Chem.* 118(1): 61-69.

67. Kitchen, J. R. and Cysyk, R. L. 1995. Synthesis and release of hyaluronic acid by Swiss 3T3 fibroblasts. *Biochem. J.* 309(2): 649-656.

68. Kujawa, M. J. 1986. Hyaluronic acid bonded to cell culture surfaces inhibits the program of myogenesis. *Dev. Biol.* 113(1): 10-16.

69. Laurent, A. E., Loof, L., Nyberg, A., Schroder, T. 1985. Increased serum levels of hyaluronate in liver disease. *Hepatology.* 5(4): 638-642.

70. Laurent, T. C., Hellsing, K., Gelotte, B. 1964. Cross linked gels of hyaluronic acid. *Acta. Chem. Scand.* 18: 274-275.

71. Laurent, T. C. and Fraser, J. R. 1992. Hyaluronan. *FASEB J.* 6: 2397-2404.

72. Laurent, T. C. 1987. Biochemistry of hyaluronan. *Acta. Otolaryngol. (Stockh) suppl.* 442: 7-24.

73. Laurent, T. C. 1998. *The Chemistry, Biology and Medical Applications of Hyaluronan and its Derivatives.* p. 43-65. Portland Press, London, England.

74. Lowry, K. M. and Beavers, E. M. 1994. Thermal stability of sodium hyaluronate in aqueous solution. *J. Biomed. Mater. Res.* 28(10): 1239-1244.

75. MacLennan, A.P. 1956. The production of capsules, hyaluronic acid and hyaluronidase by group A and group C streptococci. *J. Gen. Microbiol.* 14(1): 134-142.

76. Madaghiale, M., Conversano, F. and Mele, G. 2004. Cellulose derivative hyaluronic acid-based microporous hydrogels crosslinked through divinyl sulfone (DVS) to modulate equilibrium sorption capacity and network stability. *Biomacromolecules.* 5(1): 92-96.

77. Manfredini, R., Cavallera, V., Marini, L. and Donati, G. 1983. Mixing and oxygen transfer in conventional stirred fermentors. *Biotechnology and Bioengineering.* 25(12): 3115-3131.

78. Manna, F., Dentini, M. and Desideri, P. 1999. Comparative chemical evaluation of two commercially available derivatives of hyaluronic acid (Hylaform from rooster combs and Restylane from *Streptococcus*) used for soft tissue augmentation. *J. Eur. Acad. Derm. Vener.* 13(3): 183-192.

79. Manuskatti, W. and Maibach, H. I. 1996. Hyaluronan and skin: Wound healing and aging. *Int J Dermatol.* 35(8): 539-544.

80. Mast, B.A., Diegelmann, R. F., Krummel, T. M., Cohen, I. K. 1992. Scarless wound healing in the mammalian fetus. *Surgery Gynecol. Obstet.* 174(5): 441-451.

81. Mathews, M. B. and Decker, I. 1977. Conformation of hyaluronate in neutral and alkaline solutions. *Biochim. Biophys. Acta.* 498: 259-263.

82. Meyer, K. and Palmer, J. W. 1934. The polysaccharide of the vitreous humor. *J. Biol. Chem.* 107: 629-634.

83. Meyer, K. 1947. The biological significance of hyaluronic acid and hyaluronidase. *Physiol. Rev.* 27: 335-359.

84. Meyer, K. and Chaffee, E. 1940. Hyaluronic acid in the pleural fluid associated with a malignant tumor involving the pleura and peritoneum. *J. Biol. Chem. Mar.* 133(1): 83-91.

85. Milas, M., Rinaudo, M., Roure, I., Phillips, G. O., Williams, P. A. 2001. Comparative rheological behavior of hyaluronan from bacterial and animal sources with cross-linked hyaluronan (hylan) in aqueous solution. *Biopolymers.* 59(4): 191-204.

86. Miller, D. and Stegmann, R. 1980. Use of Na-hyaluronate in anterior segment eye surgery. *J. Am. Intraocul Implants Soc.* 6(1): 13-15.

87. Nakatani, H. 2002. Monte Carlo simulation of hyaluronidase reaction involving hydrolysis, transglycosylation and condensation. *Biochem. J.* 365(3): 701-705.

88. Nielsen, F. F. and Karring, T. 1992. Biodegradable guide for bone regeneration: polyurethane membranes tested in rabbit radius defects. *Acta orthop. Scand.* 63: 66-69.

89. Nimrod, A., Greenman, B. and Kanner, D. 1988. Method of producing high molecular weight sodium hyaluronate by fermentation of *Streptococcus*. U. S. patent. No. 4780414.

90. Norton, S., Vuilleumard, J.C. 1994. Food bioconversions and metabolite production using immobilized cell technology. *Critical reviews in biotechnology.* 14(2): 193-224.

91. Ogston, A. G., Preston, B. N. and Wells, J. D. 1973. On the transport of compact particles through solutions of chain-polymers. *Proc. Roy. Soc. Lond. A.* 333: 297-309.

92. Oksala, O., Salo, T., Tammi, R., Hakkinen, L., Jalkanen, M., Inki, P. and Larjava, H. 1995. Expression of proteoglycans and hyaluronan during wound healing. *J. Histochem. Cytochem.* 43(2): 125-135.

93. Oliferenko, S., Kaverina, I., Small, J. V., Huber, L. A. 2000. Hyaluronic Acid (HA) Binding to CD44 Activates Rac1 and Induces Lamellipodia Outgrowth. *J. Cell Biol.* 148(6): 1159-1164.

94. O'Regan, M., Martini, I., Crescenzi, F., DeLuca, C. and Lansing, M. 1994. Molecular mechanisms and genetics of hyaluronan biosynthesis. *Int. J. Biol. Macromol.* 16(6): 283-286.

95. Parekh, S., Vinci V.A., Strobel, R.J. 2000. Improvement of microbial strains and fermentation processes. *Applied Microbiology and Biotechnology.* 54(3): 287-301.

96. Prehm, P. 1984. Hyaluronate is synthesized at plasma membranes. *Biochem. J.* 220(2): 597-600.

97. Prehm, P. 1983. Synthesis of hyaluronate in differentiated teratocarcinoma cells. *Biochem. J.* 211(1): 191-198.

98. Prisell, P. T., Camber, O., Hiselius, J. and Norstedt, G. 1992. Evaluation of hyaluronan as a vehicle for peptide growth factors. *Cat. Inist.* 85: 51-56.

99. Radaeva, I. F., Kostina, G. A. and Zmievskii, A. V. 1996. Hyaluronic acid : biological role, structure, synthesis, isolation, purification, and applications. *Appl. Biochem. Microbiol.* 33: 111-115.

100. Rapport, M. M., Weissman, B., Linker, A. and Meyer, K. 1951. Isolation of a crystalline disaccharide, hyalobiuronic acid from hyaluronic acid. *Nature.* 168: 996-997.

101. Saettone, M. F., Giannaccini, B., Teneggi, A., Savigni, P., Tellini, N. 1982. Vehicles effects on ophthalmic bioavailability: the influence of different polymers on the activity of pilocarpine in rabbit and man. *J Pharm Pharmacol.* 34(7): 464-466.

102. Sato, H., Takahashi, T., Ide, H., Fukushima, T., Tabata, M., Sekine, F., Kobayashi, K., Negishi, M., Niwa, Y. 1988. Antioxidant activity of synovial fluid, hyaluronic acid, and two subcomponents of hyaluronic acid. Synovial fluid scavenging effect is enhanced in rheumatoid arthritis patients. *Arthritis Rheum.* 31(1): 63-71.

103. Scott, J., Heatley, F. and Hull, W. 1984. Secondary Structure of Hyaluronate in Solution. *Biochem. J.* 220: 197-205.

104. Scott, J. E. 1998. Secondary and tertiary structures of hyaluronan in aqueous solution. Some biological consequences. <http://www.glycoforum.gr.jp/science>.

105. Scott, J. E., Cummings, C., Brass, A. and Chen, Y. 1991. Secondary and tertiary structures of hyaluronan in aqueous solution, investigated by rotary shadowing-electron microscopy and computer simulation. *Biochem. J.* 274: 699-705.

106. Scott, J. E. and Tigwell, M. J. 1975. The influence of the intrapolymer environment of periodate oxidation of uronic acid in polyuronides and glycosaminoglycuronans. *Biochem. Soc. Trans.* 3: 662-664.

107. Scott, J. E., Presti, D. 1994. Hyaluronan-mediated protective effect against cell damage caused by enzymatically produced hydroxyl radicals is dependent on hyaluronan molecular mass. *Cell Biochemistry and Function.* 12(4): 281-288.

108. Sharif, M., George, E., Shepstone, L., Knudson, W., Thonar, E. J., Cushnaghan, J., Dieppe, P. 1995. Serum hyaluronic acid levels as a predictor of disease progression in osteoarthritis of the knee. *Arthritis and Rheum.* 38(6): 760-767.

109. Singla, S. K. 1987. Nomenclature of hyaluronic acid. *Biochem. J.* 242: 623.

110. Smedsrod, B. 1991. Cellular events in the uptake and degradation of hyaluronan. *Adv. Drug. Deliv.*

Rev. 7(22): 265-278. 111. Stamenkovic, I. and Aruffo, A. 1994. Hyaluronic acid receptors. *Methods in Enzymology*. 245: 195-216. 112. Stoolmiller, A. C. and Dorfman, A. 1969. The Biosynthesis of Hyaluronic Acid by *Streptococcus*. *J. Biol. Chem.* 244: 236-246. 113. Tammi, R., Ripellino, J. A., Margolis, R. U., Tammi, M. 1988. Localization of epidermal hyaluronic acid using the hyaluronate binding region of cartilage proteoglycan as a specific probe. *J Invest Dermatol.* 90: 412-414. 114. Tammi, R. and Tammi, M. 1998. Hyaluronan in the epidermis. *Glycoforum: Science of Hyaluronan Review Series*. <http://www.glycoforum.gr.jp/science>. 115. Tokita, Y. and Okamoto, A. 1995. Hydrolytic degradation of hyaluronic acid. *Polym. Degrad. Stab.* 48(2): 269-273. 116. Tolg, C., Poon, R., Fodde, R., Turley, E. A., Alman, B. A. 2003. Genetic deletion of receptor for hyaluronan-mediated motility (Rhamm) attenuates the formation of aggressive fibromatosis (desmoid tumor). *Oncogene*. 9(22): 6873- 6882. 117. Tuhkanen, A. L., Tammi, M. and Tammi, R. 1997. CD44 substituted with heparan sulphate and endo- beta- galactosidase- sensitive oligosaccharides: A major proteoglycan in adult human epidermis. *J Invest Dermatol.* 109: 213-218. 118. Wahler, D., Reymond, J. L. 2001. Novel methods for biocatalyst screening. *Current Opinion in Chemical Biology*. 5: 152-158. 119. Weigel, P. H., Hascall, V. C. and Tammi, M. 1997. Hyaluronan synthases. *J. Biol. Chem.* 272(22): 13997-14000. 120. Weigel, P. H., Forst, S. J., LeBoeuf, R. D., McGary, C. T. 1989. The specific interaction between fibrin(ogen) and hyaluronan: possible consequences in haemostasis, inflammation and wound healing. *Ciba. Found. Symp.* 143: 248-261. 121. West, D. C., Hampson, I. N., Arnold, F., Kumar, S. 1985. Angiogenesis induced by degradation products of hyaluronic acid. *Science*. 228(4705): 1324-1326. 122. Woolcock, J. B. 1974. The capsule of *Streptococcus equi*. *J. Gen. Microbiol.* 85(2): 372-375. 123. Xuejun, X., Netti, P. A. 2004. Preparation and characterization of a hydrogel from low-molecular weight hyaluronic acid. *Journal of Bioactive and Compatible Polymers*. 19(1): 5-15. 124. Yeung, B. and Marecak, D. 1999. Molecular weight determination of hyaluronic acid by gel filtration chromatography coupled to matrix-assisted laser desorption ionization mass spectrometry. *J. Chromatogr A*. 852(2): 573-581. 125. Yokotsuka, T. 1985. Traditional fermented soybean foods. In: *Comprehensive Biotechnology the principles, applications and regulation of biotechnology in industry*. p. 395-427. Pergamon press. London.