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Contribution of Polymeric Supports to the Development of Tissue Engineering

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Abstract: Tissue Engineering is an emerging discipline based on the concept of the rational design and fabrication of living tissues and organs for repair and replacement. The present article deals with the criteria for the selection of polymeric supports necessary for the growing and multiplication of cells responsible of the regenerative tissue. Criteria of biocompatibility, biodegradability and non toxic character of the degradation products are considered and the chemical structure and physical-chemical properties and morphology of natural and synthetic polymeric systems are described. The experimental "in vivo" study of the regeneration of sciatic nerve in rats with a guided experimental device is presented. The activation of regeneration by the sustained release of growth hormone from a slow soluble vinyl pyrrolidone-hydroxyethyl methacrylate copolymer hydrogel is shown on the basis of histopathological analysis.

Introduction

Tissue engineering is a growing multi disciplinary research field based on the regeneration of damaged organs or tissues by applying principles of transport engineering, surface reaction phenomena, as well as analytical methods to study the biological processes associated to the development and reparation of biological tissues (1). The molecular structure of substrates and their relationship with the induced biochemical reactions are also important aspects to be considered and studied, when living cells and their respective extracellular components participate in the development of devices which stimulate the regeneration of damaged organs or tissues (2, 3).

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Devices are developed based on four different strategies that uses polymeric systems from natural or synthetic origin:

-Substitution by using biostable or biodegradable materials as in heart valves of oscillating disks which are coated with a graphite layer that is a perfectly polished surface in contact with blood, or vascular grafting of Goretex (porous poly-tetrafluoroethylene) used as artificial blood vessels which can induce the formation of endothelial cells.

-Xenografting obtained from animal tissues which are stabilized by chemical treatments in order to avoid possible immunological reactions to foreign bodies, as in the case of aortic porcine valves treated with glutaraldehyde.

-Cells encapsulation in diffusion devices, micro-capsules or resorbable substrates, as for osteoblasts cultures applied to repair bone tissues.

-"In situ" application of specific substances known as growing factors, which combine with adequate scaffolds, can be applied as control release systems in part of the human body where are required, as the TGB-∃ factor with collagen which stimulate the healing process of wounds and burns.

Every system applied as scaffold in tissue engineering allows three-dimensional cell growing and obtain an extracellular matrix that must posses specific characteristics to achieve cellular nutrition, division, multiplication, generation, arrangement and consolidation of the tissue. The actual premise takes into consideration the biocompatibility-biodegradation duality.

Figure 1.- Factors affecting the quality of Polymer systems for tissue engineering

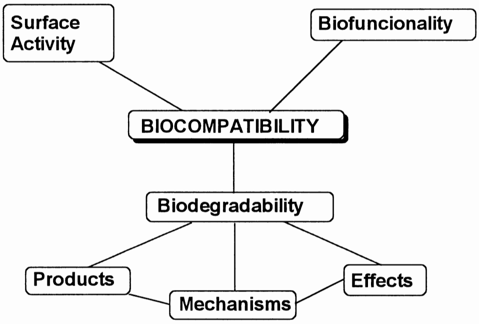

Figure 1 shows that the biocompatibility is much related to the surface activity as is the responsible of the growing system constituted by polymer/cell/extracellular matrix or polymer/growing factor, which depends on the acceptance or rejection from the patient. The systems must have also excellent biofunctional characteristics as scaffolds to give enough stability and integrity, to maintain optimal conditions during the regeneration process, as well as a biodegradable character in the manner that when the scaffold mission has been completed, the systems must be biodegraded or bioresorbed , by mechanisms that should be known in terms of degradation products, local and systemic effects and degradation rate.

The significant importance is the relationship between the cellular behaviour and the characteristics of the surface of the biomaterial scaffold or the interphase cells-support. As it is shown schematically in Figure 2, cells may interact specifically or non-specifically with the surface of biomaterial scaffolds with clear effects on the proliferation mechanism and inductive processes in the culture medium. The physical characteristics of the supports are important, since the response of cells can be different when the biomaterial is rigid or soft, or when the scaffold present a micro or macroporous structure which allows the in growth of cells into the bulk support. However, probably one of the most appropriate way to stimulate the growing and multiplication of cells is the use of microporous gel-like polymeric systems if the macromolecular components are biodegradable or soluble in the physiological medium and if they present the adequate functional components to stimulate the culture of cells. This is the case of highly hydrated hydrogels charged with active compounds like grow factors.

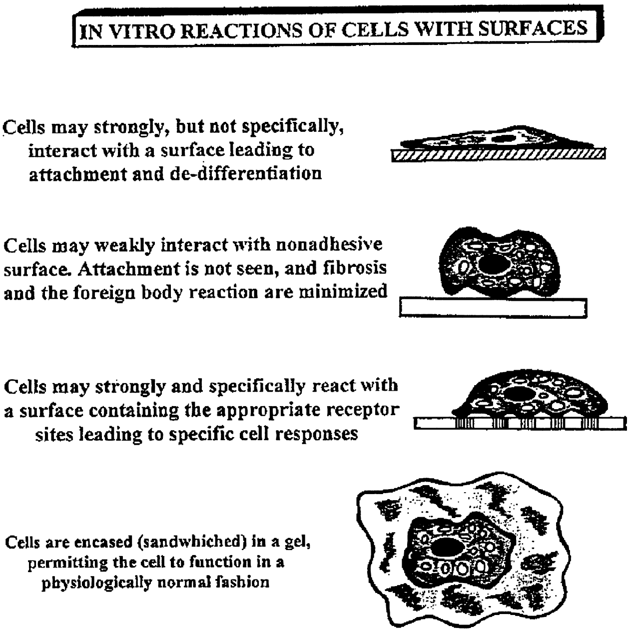

Figure 2.- Interactions of cells with surface and polymer biomaterials.

In this work we revise the principles of design and development of polymeric systems to be applied in tissue regeneration by either cell culture or direct application in the human body, or by incorporation of growing factors. The polymers are classified as: natural origin and their respective modifications, and synthetic polymers.

Natural polymers and modifications

Natural polymers based on proteins such as collagen, albumin, silk or gelatine are being modified by different methods in order to improve aspects as mechanical resistance, degradation rate and consequently their resorption in tissue regeneration, cell growing and control release processes. The most typical reactions of these natural polymers are crosslinking by using glutaraldehyde obtaining polymers with a low crosslinking degree, which can be moulded, controlled in density and porosity, as used in applications such as artificial skin (4,5). Systems as porous sterilized crosslinked collagen-hialuronic acid with fibroblasts and keratinocites from a patient, are used to prepare synthetic skin which in a few weeks present a surface with excellent cell multiplication to achieve an epidermic regeneration (6, 7), followed by the biodegradation and resorption of the polymeric system. Crosslinking these systems with poly-ethylene glycol with chemically active extremes, the hydrolytical stability is increased, which in some cases is required (8) also with the incorporation of biologically active components such as growing factors or cytokines, which are released at the same time that the polymer is degraded.

Proteins have been also modified using acylating agents such as acid chlorides, sulphonyl chlorides and anhydrides of carboxylic acids with ethylene groups which can be crosslinked by photochemical activation as has been performed in ophthalmologic applications (9). Silk proteins with bioactive peptide sequences are being produced by using genetic engineering techniques leading to materials that can be used as coatings, as fibres and co-extruded with other polymers to attain knitted meshes that can be applied as scaffolds in tissues regeneration, showing excellent mechanical properties over long periods of time (10). Elastin hydrophilic films with good mechanical stability, crosslinked by ∃ or (radiation and possessing a biomimetic character, can be also applied in the same manner as scaffolds (11).

Polysaccharides with basic character supply materials much used in this field, which are susceptible of chemical modifications. Glucosaminoglycanes are interesting systems applied as scaffolds in terms of friction resistance and loads dissipation, as well as in high hydrophilic character which make them applicable in biodynamic processes. In that sense, hialuronic acid, condroitin sulphate, chitin and chitosan are easily modified by desacetylation to obtain free amino groups and by crosslinking reactions with poly (ethylene glycol) with reactive end groups, to prepare hydrogels with high hydration degrees (12).

Polysaccharides with acidic character such as carboxymethylcellulose, carboxymethylchitin and carboxymethylstarch have been modified by different techniques using their biodegradable character and excellent biocompatibility, to be applied as biologically active supports of growing factors as well as scaffolds in cell proliferation processes, as in the case of blends with gelatine with incorporated acrylic groups which can be photochemically crosslinked to obtain hydrogels with excellent properties (13).

Crosslinked polysaccharides can be performed by using ionic interactions of the macromolecular chains which are positively or negatively charged. For example, when a sodium alginate solution is placed in contact with divalent salts (calcium, magnesium or barium) a polymeric chelate is formed by precipitation. These kind of polymeric complexes are applied to encapsulate cells to protect them from an immunological point of view as in the case of the encapsulation of pancreatic islets in calcium alginate-polylisine systems, which work to maintain the active production of insulin by these cells known as artificial pancreas for diabetic patients (14).

Polyionic complexes (PIC) are formed from the reaction of polyelectrolytes with different charges that can give capsules or fibres. A typical system is constituted by gelanes which chemical structure is an anionic polymer of [(3)-∃-D-glucose-(1,4)-∀-L-ramose-(1)] units with chitosan. They present free carboxylic groups with a 3-6 % of O-acetyl groups as sodium or potassium salts, which directly interact with the free amino groups of chitosan. After a thermal treatment at 50°C, gelified and mechanically stable beads are obtained which inner part is constituted by chitosan whereas its outer by a film of gelane (15, 16). This product can be co-extruded to make fibres and meshes with hydrophilic and biodegradable characteristics applied as supports in tissue regeneration processes. Our research group has recently studied the ionic chelate formation of acrylic polymers with salicylic acid side groups, with high molecular weight chitosan, obtaining systems with good stability, with a high hydration degree dependent on the pH, and with the ability of releasing the salicylic residue at long periods of time, which is an interesting characteristic to be applied as support in cell regeneration and cell growing processes (17). Figure 3 shows a liophilized porous chitosan-polyacrylic complex system with a high degree of porosity in the dry state. This system is able to absorb culture medium, water or physiological fluids up to high degrees of hydration with a relatively good stability, offering a convenient support for cell growing and proliferation. In addition the system is biodegraded by hydrolytical or enzymatic degradation reactions giving rise to low molecular weight non-toxic products that are metabolised or cleared from the human body without side reactions. The hydrolytical stability of the chitosan-polyacrylic complexes depends on the molecular weight of the polysaccharide chains and the polyacrylic content. The more the content of polyacrylic chains and higher the molecular weight of chitosan, the more stable the complex system results, with periods of application between one and eight weeks. Crosslinking reactions involving covalent and ionic complex formation were performed to design microcapsules and hydrogels of sodium alginate/poly-(vinyl alcohol) by photochemical “in situ” activation obtaining systems with good mechanical resistance and permselectivity properties (18).

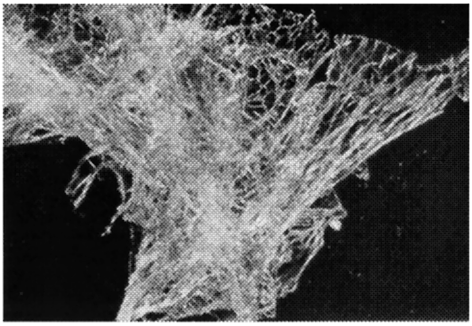

Fig 3.- Porous structure of a fibrilar semiinterpenetrating network of chitosan and polyacrylic derivative of salicylic acid

Synthetic polymers

The most important aspect to consider in the design of biodegradable synthetic polymers is the chemical functionality of the organic groups susceptible to hydrolytical processes in the physiological medium at pH = 7.0-7.4 (19). In that sense, Figure 4 shows the functional groups susceptible to hydrolysis in which carbonate, anhydride and ester are the most sensible whereas amide, orto-esters and urethane are the least ones. Factors as hydrophilic character, crystallinity, morphology and shape of the designed supports also influence on the hydrolytical behaviour of synthetic polymers.

Poly (D, L-lactic acid) PLA, poly (glycolic acid) PGA and copolymers with different compositions are the systems most applied as scaffolds in tissue engineering to produce cell regeneration and formation of extracellular matrices. The most favourable characteristic of these polymers is their biodegradable and biocompatible character as their degradation products are glycolic acid and lactic acid which are eliminated through the metabolic Krebs

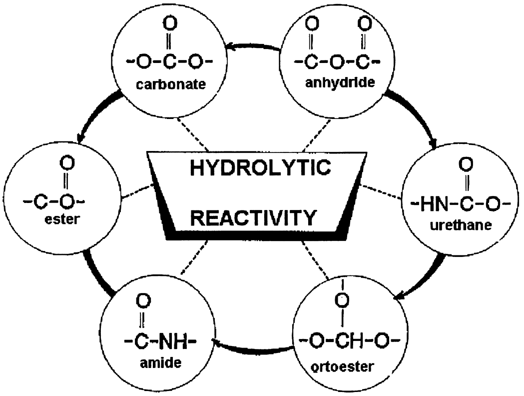

Fig 4.- Hydrolytic reactivity of organic functional groups.

cycle as carbon dioxide and water (19). Their mechanical properties and hydrolytical behaviour depend on the molecular weight which must be high to achieve mechanical and hydrolytical stability while the regeneration in taking place. Homopolymers and copolymers are synthesized by ring opening polymerization by using catalysts like zinc chloride or zinc oxide, being the most used tin 2-ethyl hexanoate. Lactide and glycolide copolymers provide a great variety of copolymer in composition, and microstructure from block to random copolymers being flexible or rigid systems with different degrees of crystallinity (20). All these properties have been used to develop porous systems which have been demonstrated excellent efficiency as scaffolds in the regeneration of connective, bone and cartilaginous tissues, even of peripheral nerves. Supercritical technology has been applied to the preparation of porous and encapsulated systems by using carbon dioxide as solvent at high pressures. Figure 5 shows microporous systems with interconnected holes of poly (lactide) and poly (D, L-lactide-co-glycolide) copolymer systems and the surface after two months of immersion in phosphate buffered solution (pH = 7.4) at 37 °C. The mechanical stability is kept during at least three months, offering an excellent support for skeletal cells proliferation which opens interesting expectations for the regeneration of bone tissues and cartilages, being materials with the absence of toxic effects as carbon dioxide is used as solvent.

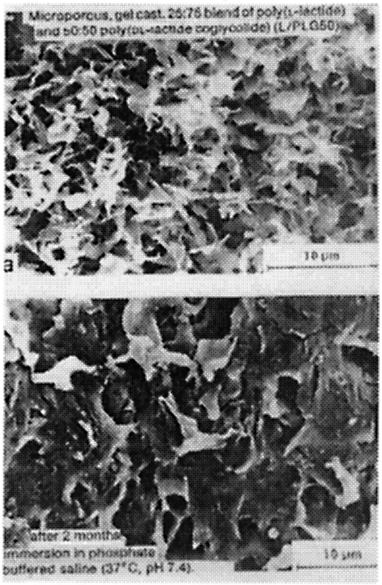

Fig 5.- Polymeric biodegradable scaffold with mechanical stability for culture of bone cells.

Poly (γ-caprolactone) is another synthetic polymer used in the design of micro particles for the release of drugs or growing factors, which is a semicrystalline system, that has also been copolymerized with D, L-lactide (21, 22) increasing the flexibility and the capability to fabricate fibres with improved adhesion and cell proliferation. Figure 6 shows the SEM micrographs of biodegradable poly (glycolic acid) fibres with a 13 µm diameter which are coated by condrocites after 3 days of culture, the cell proliferation and the production of the extracellular matrix being generated in a reasonable time for the tissue to grow and the polymer to be degraded (23).

Several copolymer modifications based on glycolic and lactic acids have introduced hydrophilic components which modify the hydrolytical behaviour as in the case of terpolymers of D, L-lactide, glycolide and γ-caprolactone with incorporated long segments of poly (ethylene glycol), using their activated chain ends as initiators of the ring opening polymerization of the three mentioned components (24). In a similar manner, tri-block copolymers poly (ethylene glycol)-poly (propylene glycol)-poly (ethylene glycol), known as "Pluronic", have been synthesized to control and modulate their hydrophilic character.

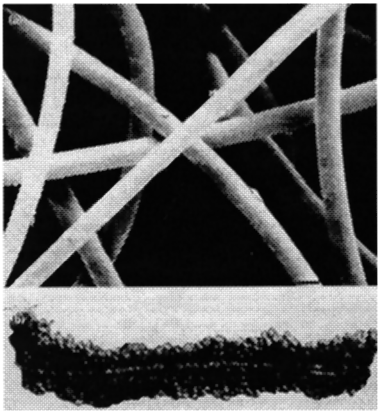

Fig 6.- Non-woven fabrics of poly(glicolide) for the culture of condrocites according to ref. 23.

Poly-dioxanone is a biodegradable poly-ester-ether prepared also by ring opening polymerization using organometallic catalysts, with applications in the biomedical field as resorbable pins, sutures and also as porous scaffolds (25). Flexible systems can be prepared by copolymerization with trimethylene carbonate (26) with excellent mechanical properties as monofilament fibres (9). Porous systems can be also prepared by using viscous solutions of polymer in volatile solvents passed through a nozzle of small diameter (200-300 μm) over a rotatory thin rod which forms a porous network when the solvent is evaporated.

Poly- anhydrides of carboxylic acids have resulted interesting biomaterials with a controlled hydrophilic character and relatively reduced hydrolytical resistance, which can be modulated by combining the length of the aliphatic chain that separates the anhydride groups. The higher the length between groups the lower the hydrophilic character and polarity and consequently the slower the degradation rate will be. Poly-anhydrides are prepared by an initial step in which pre-polymers are obtained by reaction between diacids as fumaric, succinic or sebasic acids, with acetic anhydride, and a second step in which they are polymerized at high temperature. Systems developed by Langer et al. are being applied in the controlled release of chemotherapeutic agents as implants in cerebral tumours, and are registered with the trade name "Gliadel" (27, 28). Biodegradable elastomeric thermoplastics based on block copolymers of poly-anhydrides and poly (ethylene glycol), present good properties to form flexible and mechanically stable fibres which are biodegraded by surface erosion as are highly hydrophobic materials (29).

Recently Mikos et al. have prepared polymers of poly (propylene glycol fumarate) which present double bonds in the main chain, that can be polymerized by free radical mechanism in physiological conditions. These materials are incorporated to auto curable systems or cements with growth factors as growing hormone GH, or morphogenetic protein BMP, to stimulate the tissue regeneration in bone cavities or even of connective tissues. These systems are biodegradable and can be mixed with acrylic or vinyl monomers to obtain hydrophilic or hydrophobic crosslinked matrices, as they have been performed by polymerizing methyl methacrylate, MMA, in the presence of poly (propylene glycol fumarate) and growth factors, initiated by the redox system composed by amine/peroxide, obtaining cured materials which release the bioactive component over long periods of time, followed by a slow biodegradation.

One interesting design of polymeric supports for regenerative process activated by hormones and growth factors, is based on the application of hydrophilic polymeric systems that becomes soluble in the physiological medium after a slow process of hydration and disintegration. It is not necessary that these systems are strictly biodegradable if the dissolution of the polymeric chains is produced slowly. This is possible by using copolymer systems of controlled hydrophobic/hydrophilic components considering the criteria of biocompatibility and non-toxicity necessary for applications.

Recently, we have prepared soluble non-crosslinked biocompatible copolymer systems based on high molecular weight copolymers ($Mn \approx 35\text{-}40000$ Daltons) of vinyl pyrrolidone VP and 2-hydroxyethyl methacrylate HEMA, with a dissolution profile which depends on the average composition and micro-structural distribution of the monomers in the copolymer chains (30, 31). The figure 7 shows the resorption of polymeric matrices of VP-HEMA copolymer systems with different compositions. As a result of the mechanism of polymerisation, the systems have a micro-heterogeneous distribution of VP and HEMA sequences, which modulates the rate of dissolution of the chains in the physiological medium. Copolymer systems prepared with a 60 wt-% of HEMA are very stable, whereas the system with a 30 wt-% of HEMA is dissolved in a shorter time. It is important to stress that in all cases after a small initial loss of polymeric matrix, there is a slow and continuous dissolution of the polymeric components which controls the sustained release of pharmacologically active components (i.e. cyclosporine or growth hormone) (31, 32).

We have applied the copolymer system VP-HEMA 70:30 as a support of growth hormone GH for the regeneration “in vivo” of sciatic nerve in rats. Regeneration of neural fibres into distal nerve after the excision and insertion of a graft was guided by endoneural tubes of schwann cell basement membrane, and by surrounding connective tissue (33, 34).

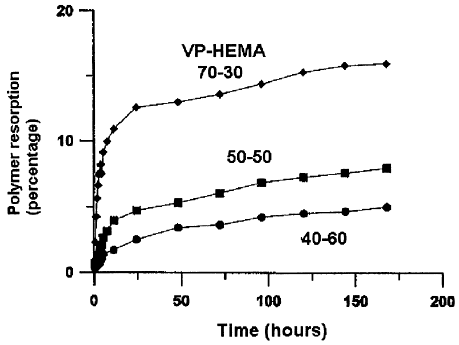

Fig 7.- Resorption rate of soluble polymeric matrix of VP-HEMA copolymers (33).

It has recognised the importance of guided and oriented regeneration for the recuperation of an adequate electrophysiological conductivity through the damaged nerve and in this sense, tubulation techniques have proven to be important tools for the study of regeneration of sciatic peripheral nerves, using biomaterials like silicone (35, 36), tubes of poly (lactic acid) and copolymers of lactic acid and glycolic acid (37, 38) and Goretex (39). We have applied the tubular model represented in Figure 8 using an external flexible tubular device of Goretex (a expanded poly-tetrafluoro ethylene with a porous morphology used in vascular surgery), charged with five or six segments of hollow fibres of poly (acrylonitrile) oriented in the longitudinal direction of the excised nerve ends and filled with a powdered VP-HEMA (70:30) copolymer system containing a 10 wt-% of growth hormone. The methodology is described in detail elsewhere (40). The use of Goretex instead of silicone tubes is justified by its good stability and biocompatibility in addition of flexibility and thickness of the wall (of 60-70 μm). The introduction of hollow fibres is related to the presence of oriented polar guides in order to promote the regeneration of nerve fibres with a longitudinal orientation in the gap profiting by the hollow fibre and the stimulation of regeneration of nervous cells by the sustained release of GH in the matrix of resorbable VP-HEMA copolymer. We consider that this is a good model for the experimental study of guided sciatic nerve regeneration. The experimental results obtained after surgical implantation of these devices into gaps of 1.0 cm of the sciatic nerve in rats, during periods of one to eight weeks, demonstrated the excellent biocompatibility of the systems and the activation of regeneration of the excised sciatic nerve with a recovery of more than 60% of electrophysiological response after eight weeks of surgical operation.

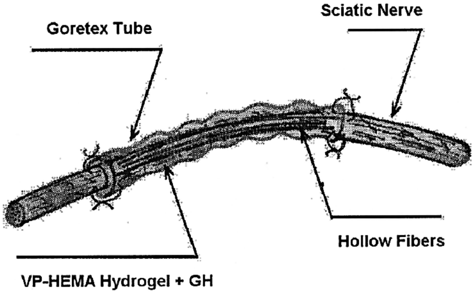

Fig 8.- Experimental model of a graft for the regeneration of sciatic nerve.

Figure 9 shows a cross-section of the system after 8 weeks, stained with haematoxylin and eosin. The black porous external cover corresponds to the Goretex tube and the internal rings and sections of the hollow fibres. Its is shown clearly the regeneration of nerve fibres in the hollow fibre as well as in the external surface of the fibre following the co-axial direction of the implanted hollow fibres.

Fig 9.- Optical micrograph (x 60) of a cross section of the sciatic nerve of rat regenerated in the tubular device with hollow fibers and hydrogel activated by GH after 8 weeks of implantation.

This is better observed in the Figure 10 recorded at higher magnification in which it is possible to show the in growth of regenerated tissue profiting by the small defects of the hollow fibres, which provides an excellent way to orient the regenerative process. Immunohistochemical experiments demonstrate that there is a good connexion between the excised nerve and the muscles which are completely destroyed when the nerve is sectioned without surgical treat.

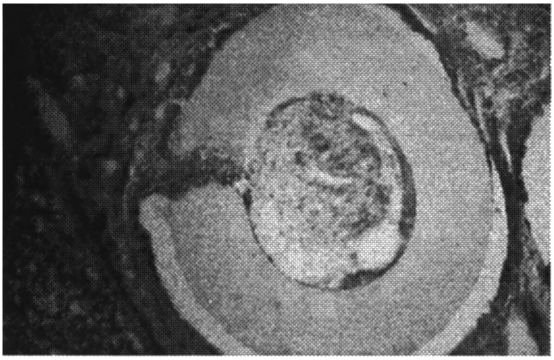

Fig 10.- Optical micrograph (x 120) of a cross sectioned of regenerated sciatic nerve after 8 weeks of implantation of the tubular device with hollow fibres and hydrogel with GH.

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