33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Next generation brain implant coatings and nerve regeneration via novel conductive nanocomposite development

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***Abstract*—Composite materials based on the coupling of conductive organic polymers and carbon nanotubes have shown that they possess properties of the individual components with a synergistic effect. Multi-wall carbon nanotube (MWCNT)/ polymer composites are hybrid materials that combine numerous mechanical, electrical and chemical properties and thus, constitute ideal biomaterials for a wide range of regenerative medicine applications. Although, complete dispersion of CNT in a polymer matrix has rarely been achieved, in this study we have succeeded high dispersibility of CNT in POSS-PCU and POSS-PCL, novel polymers based on polyprolactone and polycarbonate polyurethane (PCU) and poly(caprolactone-urea)urethane both having incorporated polyhedral oligomeric silsesquioxane (POSS). We report the synthesis and characterization of a novel biomaterial that possesses unique properties of being electrically conducting and thus being capable of electronic interfacing with tissue. To this end, POSS-PCU/MWCNT composite can be used as a biomaterial for the development of nerve guidance channels to promote nerve regeneration and POSS-PCL/MWCNT as a substrate to increase electronic interfacing between neurons and micro-machined electrodes for potential applications in neural probes, prosthetic devices and brain implants.**

Keywords- carbon nanotubes, nanomaterials, coated brain microelectrodes

I. INTRODUCTION

A new era dawns for biomaterial science as the next generation of implantable, interactive and programmable biomaterials and thus capable of seamless communication with surrounding tissues is coming to revolutionize tissue engineering and regenerative medicine. Specifically, hybrid materials that combine a wide range of desired properties and incorporate stimulatory cues, such as electrical signals, can be used to regulate cell attachment, proliferation, and differentiation. For example, electrical fields have been shown to stimulate the healing of the bone[1], cartilage[2] , skin and connective tissue[3] , cranial and spinal nerves[4] and peripheral nerves[5,6]. Consequently, researchers have sought to incorporate electrical signals directly into biomaterials since the use of electroactive materials would allow one to locally deliver an electrical stimulus at the site of the damage, while also providing a physical template for cell growth and tissue repair. To this end, studies using polymers that have been processed to display permanent changes (electrets) or to generate transient surface charges (piezoelectric materials), have demonstrated enhancement of nerve and bone cell growth in vitro and in vivo [7].

The past decade many researchers have tried to investigate the potential of carbon nanotubes as fillers in polymeric composites[10]. The electrical conductivity of insulating polymers filled with conducting particles such as carbon nanotubes, metals, carbon powder, discontinusly increases at some content of the filler. The conductivity escalates by ten orders of magnitude at the break point. The break point is associated with the affect of the filler content to conductivity range and it indicates some sudden change in the dispersing state of conducting particles[11]. The critical carbon content that correlates to the break point can differ depending on the polymer type and is likely to augment as the surface tension of the polymer rises up. The relationship between the critical carbon content on the polymer type has been an issue for decades that remains to be addressed. Since the interfacial excess energy introduced by carbon particles into the polymer matrix reaches a universal value known as Dg, the carbon nanotubes coagulate in order to avoid higher magnitudes of energy and to create networks that facilitate the electrical conduction. The alteration-state of conductivity in these composites has risen a huge interest by the scientific community and thus there is a wide range of modelling proposals for this phenomenon.

We speculate that multi-walled carbon nanotubes can be efficiently dispersed in chemical surfactants such as SDS, SBDS and the organic solvent toluene via ultrasonication etc. It is widely known, that the dispersion of CNT has never been completely successful, thus we suggest following a two-step process in order to achieve the highest dispersibility of CNTs: first, the bundles disaggregate, kinetically driven by the energy supplied to the system; second, they disperse (surfactant absorption), thermodynamically driven by the surfactant concentration. In addition, we suspect that the incorporation of CNTs into our novel polymers polyhedral oligomeric silsesquioxane integrated poly (carbonate -urea) urethane (POSS-PCU) and polyhedral oligosilsesquioxane polycaprolactone (POSS-PCL) can transform the composites into conductive polymers. These biomaterials may lead to a new therapeutical practice in regenerative medicine for brain implant coatings and nerve regenerative applications and explain why on-going medical treatments failed in regenerating nerves with elongation speed greater than 4 mm/day. Sheer polymer conduits and grafts have little impact

in nerve regeneration and this method has probably reached its zenith. In addition, the hypothesis predicts that CNT, which can reinforce electrical conductivity and thus enhance neuronal growth, may need to be combined and act synergistically with a magnetic nanoparticles strategy, in order to subtract the small concentration of CNTs out of the patient's body. The aim of this work is to demonstrate the feasibility of creating an electrically conductive polymer out of POSS-PCU and POSS-PCL which are highly insulating polymers. Once this proof-of-principle has been established, polymer optimizations can be performed; e.g., increasing the CNT concentrations or the aromatic segment length to enhance conductivity, altering the hydrophobic/hydrophilic nature of the linker to modulate degradation , and incorporating sites for attachment of biomolecules that evoke desired cellular responses. Furthermore, the present study was aimed at demonstrating the potential of the electrically conductive nanocomposite created in our labs to promote nerve cell proliferation and axon regeneration.

II. MATERIALS AND METHODS

In order to demonstrate the capability, reliability and efficiency of carbon nanotubes as nanoparticles for biomedical applications, we studied the computational mechanics approach and developed visualizations of single and multi-walled carbon nanotubes with C++ programming (described elsewhere [8]). In addition, pristine nanotubes cannot be dissolved in liquids such as water, polymer resins and most solvents due to their chemical bonding and therefore their dispersibility is remarkably low in a liquid matrix such as epoxies and other polymers. In order to achieve higher dispersibility rates in liquids, molecules or functional groups can be attached to the nanotube sidewalls via a physical or chemical procedure. This functionalization process that doesn't change significantly the desirable properties of the CNTs, enhances the formation of strong covalent chemical bonding between the filler particles and the polymer matrix along with the van der Waals physical bonds. In our study, the effective utilization of nanotubes in composite applications depends strongly on the ability to disperse the CNT homogeneously throughout the matrix without destroying the integrity of the CNT. In addition, good interfacial bonding is required to achieve load transfer across the CNT-matrix interface, a necessary condition for improving the mechanical properties for polymer composites. In this study, we address nanotube dispersion mechanisms in polymer composites by studying a model composite system in which MWCNTs are dispersed (PS) matrix with different surfactants(sodium dodecyl sulfate (SDS), sodium dodecylbenzenesulfonate (SDBS), Toluene) and their efficiency is compared [8].

Through the series of experiments that we performed we have tried to overcome some common problems that arise when MWCNTs are being used, such as the various amount of impurities, the different diameters and structures even when MWCNTs are coming from the same batch, closed tips and the inclusion of catalyst nanoparticles and internal closures so that we can achieve the best results in the conductivity measurements of our nanocomposites.

A. Synthesis of Conductive Polymer

The production of polymer POSS-PCU has been described in detail previously[9], but in brief 72g of dry polycarbonate polyol (2000Mw) and 2g of 1-(2-tran-cyclohexanediol) ethyl -3, 5, 7, 9, 11, 13, 15-isobutylppentacy-clo- [9.5.1.1. (3,9). 1(5,5). 1(7, 13)] octasiloxane were mixed in a 500 ml reaction flask with mechanical stirrer and nitrogen inlet. The polymer was heat treated (125□ C) so that the POSS cage would be dissolved and then cooled down ton 60□C. The mixture was also blended with 18.8g of flake MDI under nitrogen at 75□C for 90 minutes and cooled down to 40□C. A mixture of 2g of ethylenediamine and 0.05g of diethylamine in 80g of DMAC was added and chain extension took place. Finally, a mixture of 4g 1-butanol and 80g DMAC were added slowly to the polymer solution.

The production of POSS-PCL in brief was carried out by adding a dry polyol blend (75g) (80% wt polycaprolactonediol and 20% wt polycarbonatediol, Mw 2000) and 1g of trans-cyclohexane diolisobutyl POSS into a 250-mL reaction flask with mechanical stirrer and gas inlet. The polymer mixture is then heated to 130□C and subsequently cooled down to 60□C. Following the dissolution of POSS cage into the polyol 9.4g of flake MDI was blended under nitrogen at 75□C. Dry DMAC (100g) was added and chain extension of the prepolymer was carried out by adding 1g ethylendiamine and 0.025g of diethylamine in 80g of dry DMAC. Finally, 2g of 1-butanol in 5g of DMAC was added to the polymer solution.

After the first two months of experimentation with the POSS-PCL polymer we have decided that due to the difficulty of total dispersion of the carbon nanotubes into the polymeric matrixes it is necessary to introduce thiophene together with degradable ester linkages in order to enhance conductivity, chemical bonding and stability. Ester linkages were chosen for degradation sites because they can be cleaved by enzymes which are secreted by cells during wound repair processes, such as cholesterol esterase. The new POSS-PCL (POSS-PCL beta) after degradation is supposed to have an enhanced behavior as the remaining oligomers should be readily consumed by macrophages during wound healing response and consequently reducing chances for long term adverse responses.

MWCNTs were purchased from Nanothinx S.A. (Greece) and manufactured via chemical vapor deposition with the following specifications diameter: 25-40nm, length: ≥10μm, layers : 25-40, purity:≤ 98.5%.The samples were sonicated for a time t_s(t_s=3h), while the output power of the ultrasonicator was fixed for all experiments at 20W, thus delivering energy of 1100-1200 J/min. All samples were first heat treated in water bath at 160□C for 20min and consequently they were centrifuged at 1100g for 15 minutes to remove residuals and impurities. Experimental measurements with UV-vis spectroscopy show that dispersions are stable many weeks after preparation.

B. MWCNT/Nanocomposite nerve guides

Axonal regeneration is known to be affected by electrical charge. Electric field plays a crucial role in nerves growth as they extend in a faster rate cathodically than anodically and growth cone guidance is accelerated. In our study we have used MWCNT which were treated as described above to remove the residuals and POSS-PCL beta to create sheets for material characterization and tubes with diameters of 100nm for our intended application.

Functionalized carbon nanotubes were heated at 90□C for 15 min and then mixed with POSS-PCL beta using a homogenizer (Ultra-Turrax T25; IKA Labortechnik).

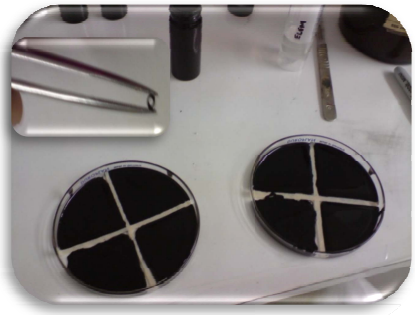

Figure 1 Tubes and casted sheets from POSS-PCL/MWCNT

The mixture was ultrasonicated for 3h with a High Intensity Ultrasonic Processor (1500 Watt Model, Sonics, USA), was cooled down to room temperature and then ultrasonicated for a cycle of 1.5 h. Following ultrasonication the mixture was kept under low air-pressure to discard the bubbles and was finally casted in the oven at 65□C over night.

C. Brain implant coatings-microelectrode coatings

Brain implant coatings need to promote the adherence between the electrode and neurons so that they enhance the signal strength as well as to offer the flexibility to add dopands to improve the conductivity and reduce impedance. In our study we have attempted to create brain microelectrode coating through layer-by-layer (LbL) deposition technique and have tested the electrical impedance. The simplicity, universality and thickness control of this technique makes it very attractive for applications in neural engineering. Initially the LbL technique has been used to assemble oppositely charged polyelectrolytes that were rapidly extending to polymeric nanocrystals and for immobilizing biological components such as DNA, viruses, proteins etc. In our study, the sequential multilayer deposition was carried out by immersing the substrate into the cationic and anionic dipping solutions, afterwards the substrate was immersed into the washing solution and was dried under the nitrogen air-flow.

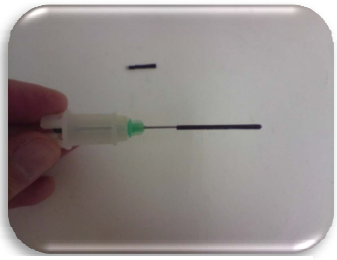

Figure 2 Coatings(d:30μm) from POSS-PCU/MWCNT

III. RESULTS

The characterisation of the surface chemistry of the carbon nanotubes and the nanocomposites were performed using Fourier Transform Infra-red Spectroscopy (FTIR), Raman Spectroscopy, UV/vis spectroscopy, Electrical Impedance, Electrochemical Impedance and 4 –Point Probe electrical testing. The imaging of the functionalised carbon-nanotubes and the conductive polymer nanocomposites was performed on TEM (transmission electron microscope), SEM (scanning electron microscope) and AFM (atomic force microscope). We have also performed viscosity measurements, tensile strain mechanical testing and contact angle measurements to gain more information about the physical and mechanical properties of the samples.

A. Nanocomposite measurements

Electrical conductivity measurements of our polymeric nanocomposites are essential for the stimulation of responsive cells. We have tested the electrical conductivity through 3 different methods: electrical impedance (samples in solid phase), electrochemical impedance (samples in liquid phase) and four point probe testing. Since plain POSS-PCU and POSS-PCL were found to be completely insulating materials our control for comparison was polypyrrole that has a conductivity that ranges from 1-100 S/cm.

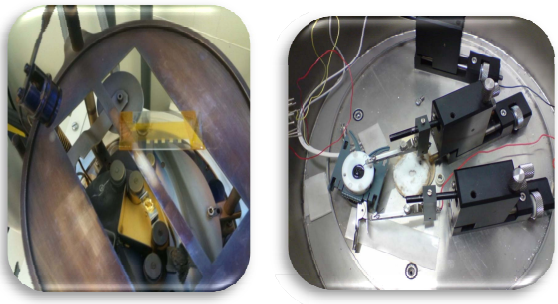

Figure 3 Au-coated diamonds (left) used on the electrochemical impedance setup (right) to test 4μl of nanocomposite for EC

Conductivity measurements delivered the following graphs and the raw data have been also processed through JVIEW software 2.8d in order to fit the semicircles with accuracy and for better analysis. The microelectrode coatings with LbL technique produced the following results.

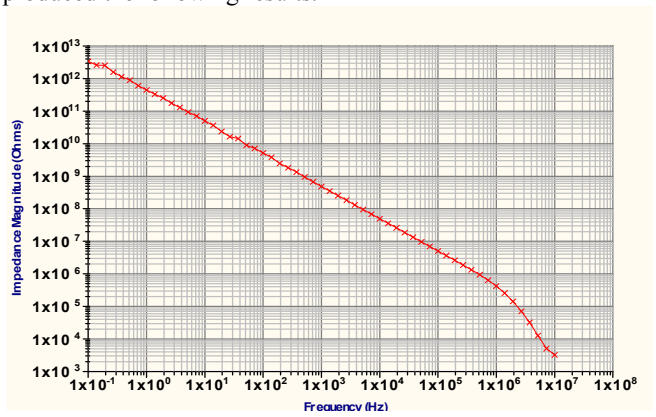

Figure 4 Electrical Impedance measurement on sheet of nanocomposite

As we can see in Fig 5 the response to the AC stimuli of varied frequencies yields a semicircular shape in the complex impedance plane plot, widely known as the Cole-Cole plot. The semicircular Cole-Cole response suggests an RC-circuit behavior and for all our nanocomposite samples we can assume that the dominant larger semicircle may result from electron conduction through MWNT-POSS interface.

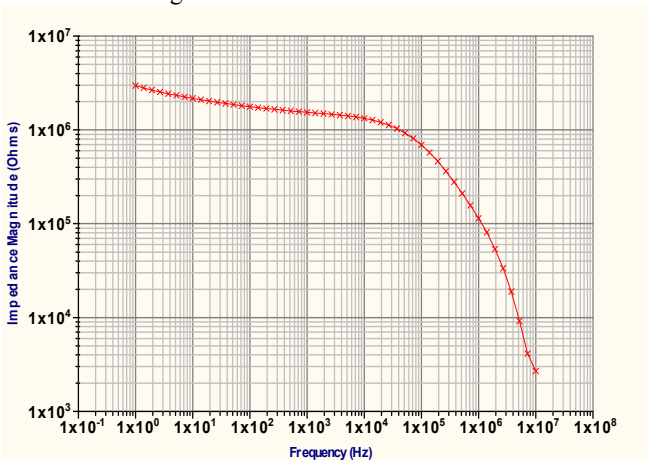

Figure 5 Electrochemical impedance measurement on nanocomposite in liquid phase

Since it is known that carboxylic functional groups are always present at the ends of carbon nanotubes we have tried to functionalize our MWCNTs by adding –COOH groups.

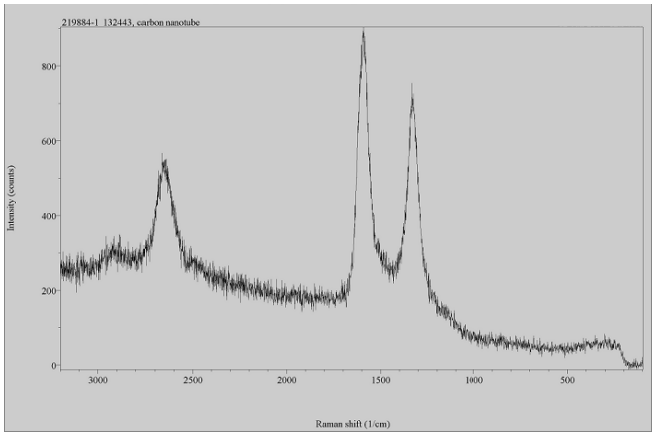

Figure 5 Raman spectrum of functionalized -COOH MWCNTs

The Raman spectrum is the plot of intensity of scattered radiation as a function of the shift between the incident and the scattered light. The main peak appears at approximately 2700 cm^{-1} with laser wavelength at 488nm. The disorder-induced D band appear at 1354.8 cm^{-1}, the tangential mode G appears at 1657.4 cm^{-1} and the second order G' band appears at 2724cm^{-1}. The feature at 1357.8cm^{-1} is the D band due to amorphous carbon while the peak 1657.4$cm^{-1}$is the G band that predominates in highly crystalline MWCNTs. The intensity relationship between D and G indicates a low crystallinity of the sample.

Morphological studies of the nanocomposites were carried out via SEM. The samples that were treated with SDS (A), toluene (B) and graphite (D) demonstrated some blobs of amorphous carbon and some thick bundles (of diameter approximately 30-40nm). The periodicity of spacing between the nanocrystals is 40±20nm. The nanocomposite with SDBS is shown to have a more even surface with smooth peak variations, that were also confirmed via the contact angle measurements where we calculated 84.25. The hydrophilicity of the polymer samples was tested as this property is a crucial factor that affects protein absorption and cell attachment. Generally speaking, wettability assays are important in polymer technology as they generate thermodynamic data The internal water contact angle of the POSS-PCU/MWCNT (SDBS) and POSS_PCL/CNT (SDBS) are close to 77□ ±2□ (mean ± standard deviation). Studies have shown that contact angles in the range of 40□-80□ maximize the adhesion of multiple cells.

The same observations can be more clearly derived from the AFM photomicrographs that give a more detailed view of the surface topography and the peaks that are associated with the different types of functionalization used.

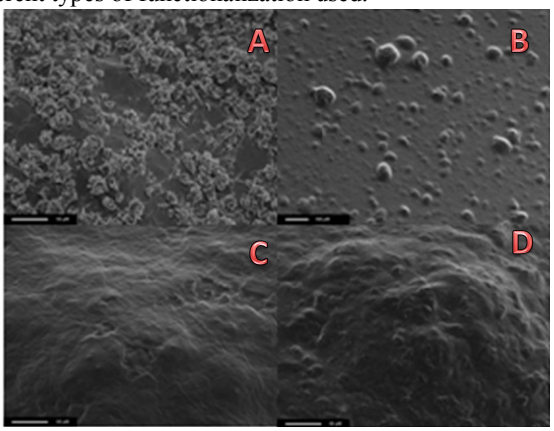

Figure 6 SEM images of nanocomposites with MWCNT functionalized with SDS (A), toluene(B), SDBS(C) and graphite(D)

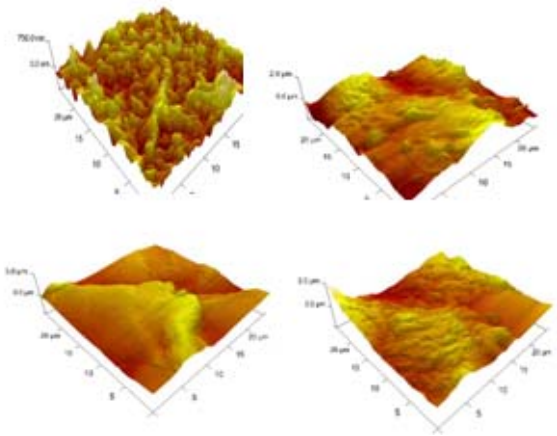

Figure 7 AFM images of nanocomposites with MWCNT functionalized with SDS (A), toluene(B), SDBS ((C) and graphite(D)

The transmission spectrum of POSS-PCU/MWNT (SDBS) ranges from 50-60% between 350 to 1850nm. The reflectance spectra has a similar wavelength for its rough and smooth surface although the smooth one is slightly more reflecting between 1200-1750nm.

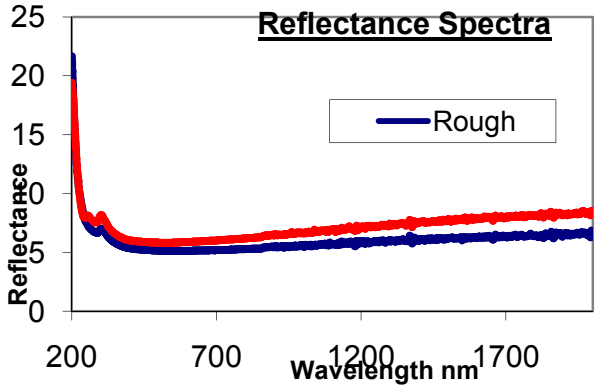

Figure 8 Reflectance Spectrum of rough and smooth surface of nanocomposite.

IV. Conclusions

The process of synthesis polymer nanocomposites is a complex task and can be achieved through three different experimental methods: mixing in the liquid state, solution-mediated processes and in-situ polymerization techniques. Each technique produces different nanocomposites as the CNTs are very susceptible (sensitive) to heating procedures and therefore the direct-melting approach is preferred as it is more commercially attractive, more versatile and less environmentally contentious than the other two. In this study we have performed the approach of mixing in the liquid state by using multi-walled carbon nanotubes with biodegradable and non-biodegradable polyurethane polymers in order to create conductive nanocomposites for the creation of nerve conduits and coatings for brain microelectrodes.

The MWCNT/polymer composites that were formed from our 4 samples (4%wt) (POSS-PCU/MWCNTs-toluene-treated, POSS-PCU/MWCNTs-SDS-treated, POSS-PCU/MWCNTs-SDBS treated and POSS-PCL/MWCNT-SDBS-treated) have unique electrical properties since the CNTs incorporated into the host matrix result in a very low percolation threshold. The incorporation of carbon nanotubes into an insulating polymer host leads to bulk conductivities that exceed the anti-static limit of 10^{-6} S/m. Although metallic and graphite particles are extensively used as conductive fillers in various shapes and sizes, carbon nanotubes allows for a low percolation threshold, high quality surface finish, robust network and good mechanical properties. In order to test this assumption we have also compared our samples with a nanocomposite filled with graphite particles and tested their electrical conductivity range. It has been evident by the electrical impedance measurements that MWCNTs are better reinforcements than graphite particles by 2 orders of magnitude. Generally speaking, the electrical conductivity of a nanocomposite describes a non-linear increase with the particle concentration, passing through a percolation threshold. In cases of low particle concentrations, the conductive filler do not form aggregates and the electrical properties of the composite are dominated by the matrix. At the percolation threshold, φ_c, the aggregates can form a three-dimensional network that can give high peaks in the electrical conductivity measurements. However, due to the insolubility of CNTs in common solvents and polymers, aggregation and dispersibility issues were faced during our attempts to create coagulated polymers. To overcome these difficulties, we have dispersed CNTs in certain polymer solutions via ultrasonication and used dry surfactants. The nanocomposites tested were formed as sheets and as coatings for brain microelectrodes. The imaging techniques revealed the roughness and the crystallization of composites created with Toluene and SDS and thus SDBS has proven to be the gold standard for our intended applications.

Acknowledgment

We would like to acknowledge Dr. Arnold Darbyshire for his valuable assistance regarding the development and the chemical synthesis of the nanocomposites and his insight.

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