Neuritogenesis and Synaptogenesis Promoted by Acetylcholine-like Motifs in Biodegradable Polymers

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Introduction: Neurotransmitters have been shown to be essential in embryonic and neonatal neuronal development. Acetylcholine (Ach) induces neurite outgrowth and may promote the formation and strengthening of synapses [1]. We hypothesize that a polymer of appropriate Ach content will promote the maximum neurite outgrowth and synapse formation. Here we present a series of new biodegradable polymers that contain varying concentrations of Ach functional groups through the polycondensation of aminoethyl acetate and diglycidyl sebacate (**Fig.1**). The concentration of the Ach moieties was adjusted by copolymerization with the inert spacer L-leucine ethyl ester.

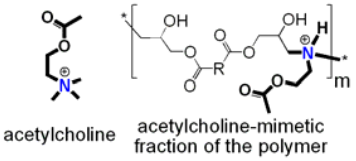

Fig. 1. Structural similarity between Ach and title polymers.

Methods: *Synthesis of monomers: (i) diglycidyl sebacate monomer.* The condensation product of allyl alcohol and sebacic acid is oxidized by *m*-chloroperoxybenzoic acid to produce said monomer. *(ii) aminoethylacetate* Aminoethylacetate hydrochloride was synthesized according to Liu *et al.*[2]. *Synthesis of Ach-inspired polymers.* The following polymers were synthesized in the presence of equimolar $Mg(ClO_4)_2$: $PSA_{100}L_0$, $PSA_{60}L_{40}$, $PSA_{40}L_{60}$, PSA_0L_{100} (A_x = %Ach, L_y = % leucine ethyl ester) *Material characterization*. The resultant polymers were characterized with FTNMR, FT-IR, DSC, and GPC. *Dorsal root ganglia (DRG) culture*. Glass coverslips were coated with the corresponding polymer solution (4.62 mg/ml) and dried overnight at 600 mTorr. DRG from 3-day old Sprague-Dawley rats were cultured on the coverslips. Neurite length was analyzed using ImagePro; synaptophysin and neurofilament staining were performed with appropriate antibodies (Biomeda Corp. and Chemicon Intl., respectively).

Results and Discussion: DRG showed significant neurite extension on $PSA_{60}L_{40}$, $PSA_{40}L_{60}$, and PSA_0L_{100} (**Fig. 2a).** DRG had few (<5 short neurites) or no neurite growth on $PSA_{100}L_0$. More neurites grew on $PSA_{60}L_{40}$ and $PSA_{40}L_{60}$, showed extensive arborization, and were longer than those on PSA_0L_{100}. Synapse formation was extensive on PSA_xL_y as indicated by synaptophysin staining (**Fig.2b**). With at least 45 of the longest neurites that were measured on each polymer, the median neurite length on $PSA_{60}L_{40}$ and $PSA_{40}L_{60}$ were comparable and considerably longer than on PSA_0L_{100}. At day 4, $PSA_{60}L_{40}$ produced neurites up to 2.8 mm (**Fig.3)**.

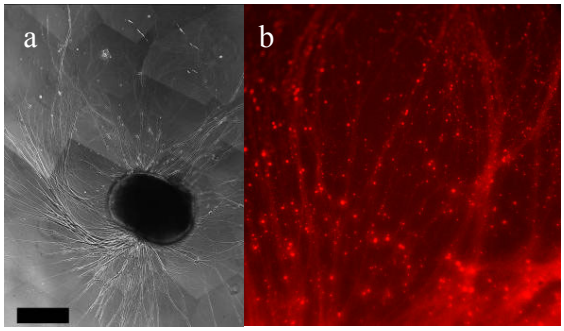

Fig. 2. Ach-mimetic polymers promote neuritogenesis and synaptogenesis. Both DRG above were grown on $PSA_{60}L_{40}$. (a) At day 8, DRG has long, branching neurites extending all around the body. Image obtained by merging multiple 100x photomicrographs. Scale bar = 1mm. (b) Synaptophysin staining of DRG grown on Ach polymer (200x). Numerous synapses formed between neurites, indicated by punctuate red spots. DRG, which express Ach receptors, might recognize the Ach-mimetic functional groups of the polymer.

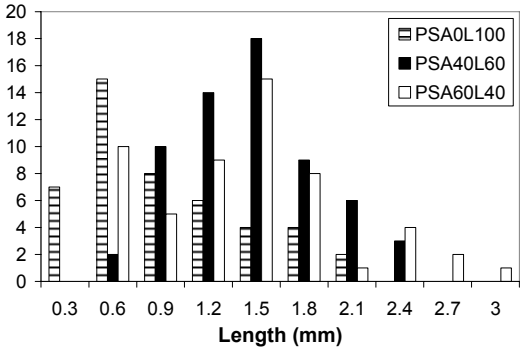

Fig. 3. Histogram of neurite lengths on DRG cultured for 4 days. DRG on $PSA_{60}L_{40}$ and $PSA_{40}L_{60}$ had median neurite lengths longer than those on PSA_0L_{100}. On $PSA_{60}L_{40}$, however, there were neurites of almost 3 mm compared to those on $PSA_{40}L_{60}$ of 2.4 mm. There were few or no neurites on DRGs grown on $PSA_{100}L_0$. There appeared to be an optimal concentration to stimulate neurite growth. Insufficient Ach led to shorter neurites while over-stimulus with too much resulted in no neurite extension.

Conclusions: We have synthesized a series of polyesters containing various concentrations of Ach-like functional groups. A 60% Ach content appeared to be the most efficient at promoting neuritogenesis and synaptogenesis. We are currently investigating the potential of this new biomaterial in an *in vivo* nerve regeneration model.

References:

1. Tata AM. J Neurosci Res 2003;73:227-234.
2. Liu J. J Org Chem 2001;66:5655-5663.