Chapter VI. Conclusions and Future Directions

IGF-I cell surface binding, in the absence or presence of IGFBP-3, is influenced by HSPG. HSPG directly regulates IGF-I cellular activation and does not appear to regulate IGF-I cell surface binding through the regulation of IGFBP-3 as previously suspected. HSPG does enhance IGF-I cell surface binding.

In this thesis, cell-free assays (Chapter II) have been used to determine:

• IGF-I and IGFBP-3 binding affinity
• IGFBP-3 and p9 HS binding characteristics
• IGF-I and p9 HS binding affinity
• IGFBP-3, p9 HS and IGF-I binding characteristics

Biodot assay experimental results showed that IGF-I and IGFBP-3 have an affinity (K_D) for each other. The binding affinity could not be used in model predictions due to the lack of quantitative data on IGFBP-3 retention on the membrane. However, results for IGF-I and IGFBP-3 cell-free binding mimic results seen with other cell-free assays presented. Thus, the Biodot assay is a valuable tool in qualitatively analyzing IGF-I and IGFBP-3 binding characteristics. Increased temperature and incubation time decreases IGF-I and IGFBP-3 binding which may be due to dissociation during the longer incubation time or an affect of temperature on IGFBP-3 retention.

p9 HS does not appear to bind IGFBP-3. In the Biodot assay, p9 HS drove IGFBP-3 from the membrane which is likely due to the higher negative charge of p9 HS. There was no change in IGFBP-3 and p9 HS binding with lengthier incubation time and an increased temperature.

The Biodot assay also demonstrated an affinity of p9 HS for IGF-I. The amount of p9 HS retained on the membrane could be quantified and therefore the binding affinity for IGF-I and p9 HS could be used for model purposes. However, due to the restriction on the p9 HS concentration by membrane saturation during the Biodot assay, binding affinity values were used only for comparison with other cell-free assays. Increased temperature and incubation time does not alter binding of IGF-I and p9 HS. IGF-I and p9 HS have a

lower binding affinity for each other than IGF-I and IGFBP-3.

IGFBP-3 increased IGF-I retention in the presence of p9 HS with the biodot assay. Due to the low concentration of p9 HS used in the Biodot assay, it is possible that there was an appreciable amount of IGF-I still in solution and the addition of IGFBP-3 helped carry any free IGF-I not bound by p9 HS to the membrane.

p9 HS slightly decreased IGF-I retention on the membrane in the presence of IGFBP-3. This suggests one or all of the following, which are important areas that should be followed up on: 1) IGF-I and IGFBP-3 can bind to the same site on p9 HS; 2) p9 HS drives IGFBP-3, and consequently IGF-I/IGFBP-3 complexes, off the membrane; 3) p9 HS prevents IGF-I binding to IGFBP-3 or releases IGF-I from IGF-I/IGFBP-3 complex.

The charcoal assay was the preferred technique for determining IGF-I and IGFBP-3 or p9 HS binding affinity. IGF-I and IGFBP-3 have a higher binding affinity (3×10^{-9} M) for each other than IGF-I and p9 HS (1.5×10^{-8} M). The binding affinities determined from this assay were used in model predictions.

Cell binding studies (Chapter IV) and mathematical models (Chapter V) have been presented to examine a number of important BAE Cell System features with regard to IGF-I surface binding:

• IGF-I/IGF-IR binding
• IGFBP-3 regulation of IGF-I cell surface binding
• p9 HS regulation of IGF-I cell surface binding
• dual IGFBP-3 and p9 HS regulation of IGF-I cell surface binding

Experimentally, it was found that IGFBP-3 decreased IGF-I BAE cell surface binding while p9 HS slightly increased IGF-I BAE cell surface binding. The presence of p9 HS countered the decreasing effect of IGFBP-3 on IGF-I BAE cell surface binding. Although preliminary experiments with labeled p9 HS and IGFBP-3 indicated little to no cell surface binding, later experiments indicated that both IGFBP-3 and p9 HS do bind to the BAE cell surface. Pre-incubation of BAE cells with either IGFBP-3 or p9 HS resulted in an increase in IGF-I BAE cell surface binding . There was a more substantial increase when cells were pre-

incubated with IGFBP-3 than p9 HS. In the absence of pre-incubation, IGFBP-3 reduced IGF-I cell surface binding. However, when IGFBP-3 was given a chance to bind to the surface first, IGF-I cell surface binding was increased. This suggests that IGFBP-3 cell surface binding plays a key role in IGF-I cell surface binding. In this study, there was no attempt to differentiate between IGF-IR and any other surface binding. In addition, there was no examination of cell activity assays which may yield differing results. There was a larger increase of IGF-I BAE cell surface binding when cells were pre-incubated with p9 HS than when p9 HS and IGF-I were added simultaneously. This also suggests that p9 HS surface binding plays a role in IGF-I surface binding. The next query is whether receptor binding is what is altered and whether or not activities such as transcytosis are influenced. Reaching the cell surface is the initial step and future work is required to follow up on what transpires next.

There is, then, room for much future experimental work. Follow-up on temperature and incubation time effects on IGFBP-3 and IGF-I cell-free experiments would be worthwhile. There are many biologically interesting experiments that could be done with regard to p9 HS regulation of IGF-I surface binding. Enzymatic digestion of p9 HS with Hep I and Hep III may change the regulatory action of p9 HS. -elimination reactions freeing the core proteins from GAG chains may also affect p9 HS activity. It has been shown with bFGF binding to vascular smooth muscle cells that the whole proteoglycan is more effective than the free GAG chains[30]. Whether this is the case with IGF-I has yet to be investigated. ^{125}I labeling of p9 HS would allow for further exploration of p9 HS BAE cell surface binding. Quantitation of IGFBP-3 and p9 HS binding affinities for their respective surface receptors would allow for future models including soluble receptor surface binding to be developed.

Complete agreement with experimental results is not obtained using our mathematical model which does not include soluble receptor surface binding. The IGF-I/ IGFBP-3 BAE Cell System Model predicted that a decrease in IGF-I cell surface binding would be seen with increasing IGFBP-3. This was confirmed with experimental results. However, the latest experimental results indicate that IGFBP-3 cell surface binding plays a key role in IGF-I cell surface binding. The addition of IGFBP-3 cell surface binding to an independent surface receptor is likely to affect model predictions. The estimates taken from the literature for receptor density and the association rate constant for IGF-I and IGFBP-3

may differ for the BAE system and may account for some discrepancies between the model and experimental results.

The IGF-I/p9 HS BAE Cell System Model predicted that there would also be a decrease in IGF-I cell surface binding which was not verified with experimental cell binding studies. Again, p9 HS surface binding does affect IGF-I surface binding, which when accounted for in the model is likely to alter results. The estimated values for receptor density and the association rate constant for IGF-I and p9 HS may account for some discrepancies between the experimental and model results.

The Complex BAE Cell System Model predicted that there would be a decrease of IGF-I cell surface binding in the presence of p9 HS. This agrees with experimental results. Experimental data showed that when IGFBP-3 (at low concentrations) and p9 HS were present together with IGF-I, there was an increase in IGF-I cell surface binding. The model did not predict an increase using the same scenario. This is more than likely due to the neglect of p9 HS and IGFBP-3 surface binding. Also, it should be noted that the model did not include the presence of alternative non-receptor binding sites which experimentally may be present.

Measurements such as receptor densities and accurate binding rate constants are important for model predictions. Future models, accounting for soluble receptor surface binding, are being developed to investigate how soluble receptor surface binding impacts IGF-I cell surface binding. It would be advantageous to quantify the binding affinities of IGFBP-3 and p9 HS to their respective surface receptors for valid model predictions. However, qualitative inclusion may indicate whether this mechanism can account for the experimental evidence.

Experimental work helps further the understanding of IGF-I cellular activation as regulated by binding proteins and proteoglycans. Developing mathematical models allows the researcher to focus on individual elements in a complex systems and gain insight on how the real system will respond to individual changes. Discrepancies between the models and the experimental data presented indicate that soluble receptor inhibition is not sufficient to account for experimental results. Ongoing experimental work within our laboratory is aimed at measuring parameters our work has indicated are important (e.g.,

binding affinity of IGFBP-3 and IGFBP-3 cell surface receptor). Mathematical models for the BAE Cell System including soluble receptor surface binding are still in the process of being developed.

Our latest experimental work has indicated that IGFBP-3 and p9 HS do bind to the surface of BAE cells. IGFBP-3 has been found to bind to a different cell surface receptor than IGF-I in Chinese Hamster Ovary (CHO) cells and has direct cellular effects that are independent of IGF-I[39]. The class of IGFBP-3 cell surface receptors, the transmembrane domain, and the capacity for cellular signaling has yet to be determined. This work indicates that this binding or its subsequent effect on IGF-I binding may be influenced by local proteoglycans. This may be an important alternative control mechanism for proteoglycans which will be examined in the future.

The possibilities for the prevention and treatment of many diseases, such as cancer, atherosclerosis and rheumatoid arthritis, continue to grow with enhanced knowledge of how growth factors function. The increased accessibility of growth factors by recombinant DNA methods grant researchers free exploration into the possible therapeutic value of growth factors and/or their antibodies. Tissue regeneration and wound healing may be refined by regulating the levels of growth factors existing in the human body. Growth factors or their manufactured counterparts may be beneficial in improving tissue function defects. This would involve an efficient ligand delivery system which would require improvements to existing pharmacological methods or the development of new delivery devices that the body will not reject. The addition of exogenous soluble receptors aimed at growth factors could aid in preventing certain diseases associated with high growth factor activity levels by interrupting regular cellular signaling pathways. Lung tumors express high levels of IGF-IR[5]. Thus, control of IGF-I levels by soluble receptors could affect IGF-I/IGF-IR binding. IGF-I/IGF-IR binding leads to activation and to IGF-I-induced receptor downregulation which may influence tumor growth. The over-expression of IGF-I in transgenic mice resulted in an increase in body weight[77] and in reverse could be helpful for this nation's increasing obesity problem. IGF-I has already been shown to increase the growth rate in dwarfism patients[78]. Amyotrophic lateral sclerosis (Lou Gehrig's Disease) may be helped by IGF-I; clinical investigations are underway[79].

The alliance of engineering analysis and molecular biology helps to clarify

significant principles relevant to the conveyance of growth factors into tissue. Awareness of the effects of individual parameters in the delivery system, made possible with mathematical models, will provide guidance and save time in the design of future therapeutics involving growth factors.