Particular poor prognosis for patients in advanced stages of solid tumours have opened the possibility that tumours include a population of cells responsible for the initiation of tumour development, growth and its ability to metastasize and reoccur. Because these cells share some similarities with stem cells, they are referred to as cancer stem cells (CSCs). CSCs possess inherent properties of self-renewal and differentiation, along with expressing certain genes related to a mesenchymal phenotype. These features favour the promotion of tumour recurrence and metastasis in cancer patients. Treatment of malignant gliomas represents one of the most formidable challenges in oncology. Despite treatment with surgery, radiation therapy, and chemotherapy, prognosis remains poor, particularly for GBM. Quiescent, or slowly proliferating, CSCs may contribute to chemotherapy resistance as this therapy acts mainly on rapidly cycling cell populations. Issues regarding CSC movement are important in neurosphere biology as cell–cell or cell–environment interactions may have significant impacts on CSC differentiation and contribute to the heterogeneity of the neurosphere. Despite the growing body of literature data on the biology of brain tumor stem cells, floating CSC-derived neurospheres have not been fully characterized from a morphological and ultrastructural point of view. Thus, to better understand the mechanisms underlying GBM CSC biology, the behavior of the CSCs was followed in living conditions by time-lapse videomicroscopy and by scanning electron microscopy (SEM). Several CSC cell lines isolated from glioblastoma patients were analyzed. The invasive potential was assessed by transwell chamber invasion assay and was compared with SEM observations carried out after 3 and 20 hours after deposition on a Matrigel® film. After 3 hours from seeding, CSC cells invaded the film organizing long cords constituted by bipolar elongated cells (Figure 1). Moreover, satellite cells with peculiar surface morphology (Figure 2) were present in all CSC lines analyzed. In particular experimental condition, CSCs formed characteristic units composed by a flat epithelioid cell housing other CSCs with globular morphology (pseudo-niche) (Figure 3). After 20 hours, CSC agglomerates displayed the surface completely covered by the Matrigel® film (Figure 4), only satellite cells lined outside the structure. The results obtained in this study indicated (i) the ability of CSC populations to self organize in ordered structures; (ii) the presence of satellite cells whose location suggest a specific role still to be clarified; (iii) the presence of pseudo-niche like structures, i.e. the pluripotentiality of the population of glioblastoma isolated stem cells.
Fig. 1: Bipolar elongated CSC cells invaded the film organizing cords.

Fig. 2: Satellite cell.

Fig. 3: Pseudo-niche constituted by flat epithelioid cell housing CSCs with globular morphology.

Fig. 4: CSC agglomerates covered by Matrigel™ film.