Selection and Enrichment of Cancer Stem Cells Utilizing Surface Structure Modification

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Cancer stem cells (CSCs) represent a subpopulation of tumor cells endowed with self-renewal, tumor initiation, disease relapse or metastasis, and resistance to radiotherapy and chemotherapy. However, the major difficulty of CSCs research is how to isolate CSCs from solid cancer and maintain their survival. Herein, binary colloidal crystals (BCCs) composed of hyaluronic acid (HA) were established and simulated with liver carcinoma CSCs microenvironments for CSCs selection and enrichment with a label-free culture system. When cells cultured on BCCs-HA system, human hepatocellular carcinoma cells (HepG2) aggregated and formed colony after 3 days culture. The expression of CSCs markers such as CD133 and CD44 of these cancer stem-like cells isolated from BCCs-HA culture system will be examined by using immunofluorescence staining. Therefore, according to the results, it is supposed that microenvironments selection and colony formation on BCCs-HA culture system may provide a label-free CSCs selection strategy and drug testing model for future biomedical applications.

Experimental design

Material

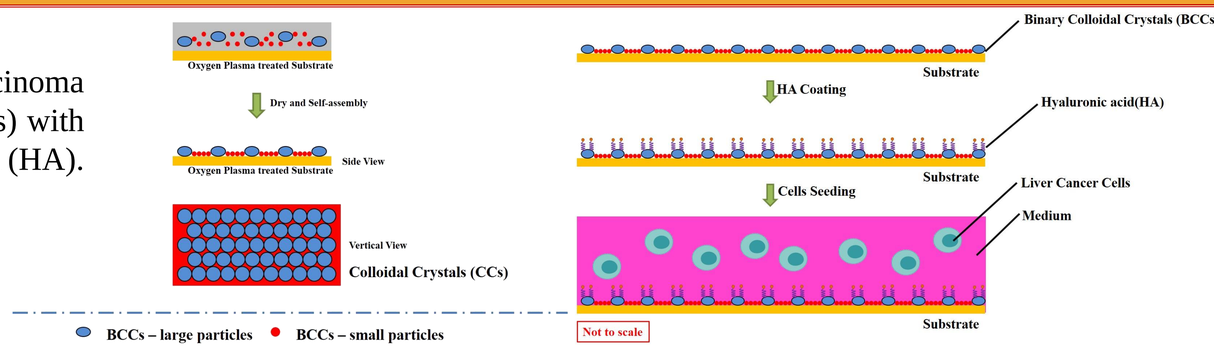
- Binary/Ternary Colloidal Crystals (BCCs/TCCs) particle
  - PSC: carboxylated polystyrene; PSN: aminated polystyrene; PSS: sulphated polystyrene; PMMA: poly(methyl methacrylate); SiC: carboxylated silica; SiN: aminated silica.
- Hyaluronic acid (HA)

Method

- To seed the human hepatocellular carcinoma cells on binary colloidal crystals (BCCs) with different concentrations hyaluronic acid (HA).

Characterization Analysis

- Material structure analysis
- Cell toxicity
- Immunofluorescence assay
- RT-PCR



Scheme 1. Illustration of the BCCs-HA structure and colonies formation types.

Material Analysis

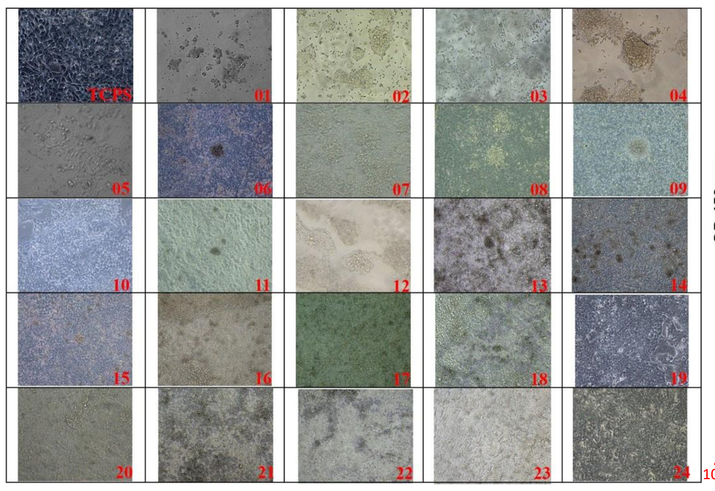


Fig 1. HepG2 cell culture on series BCCs monolayer with different sizes of large and small particles after 5 days of culture, BCCs show different size ratios (r) ranging from 0.012–0.25.

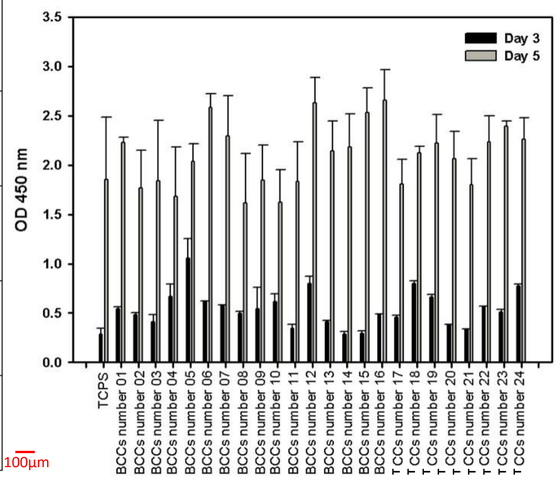


Fig 2. CCK-8 assay of HepG2 cells on BCCs monolayer.

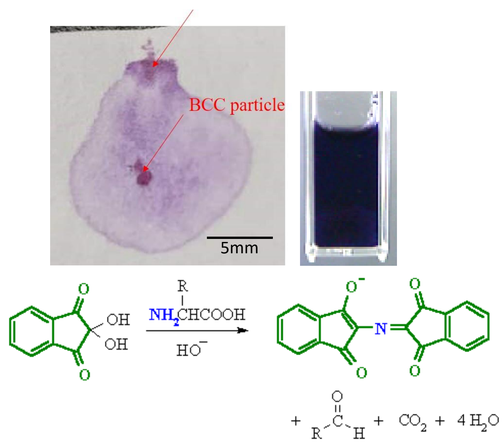


Fig 3. Ninhydrin test for amino group (-NH_2) on BCCs particles.

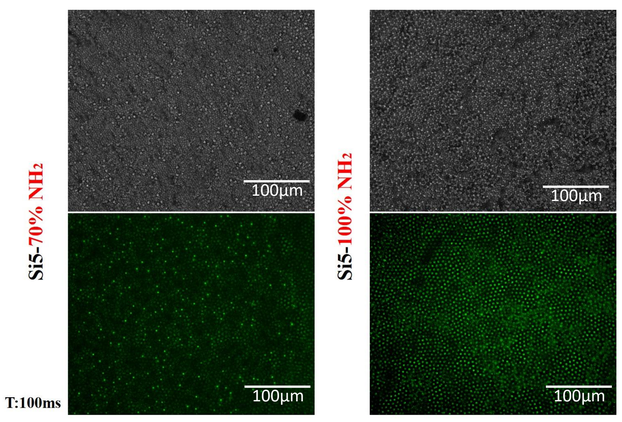


Fig 4. RGD-FITC was used to connect BCC particles instead of HA, and fluorescence intensity was used to indicate the connect proportion. The results show that the number of amine group modified particles can be used to control the HA attachment ratio.

HepG2 Sphere on BCCs-HA Surface

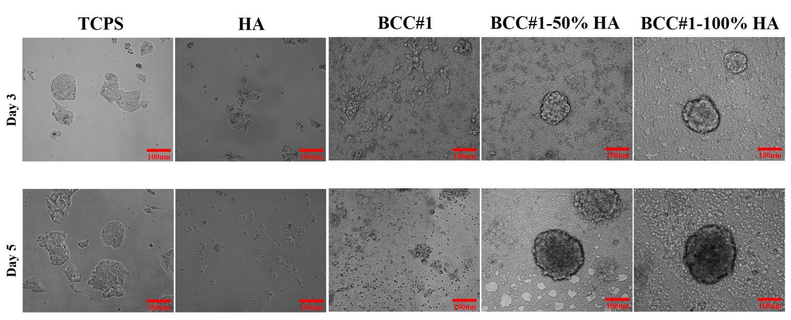


Fig 5. Phase contrast of HepG2 cell line on different type of material surface, and successfully formed cell spheres on the BCCs surface with different concentration of HA .

Sphere Number and Size Statistics

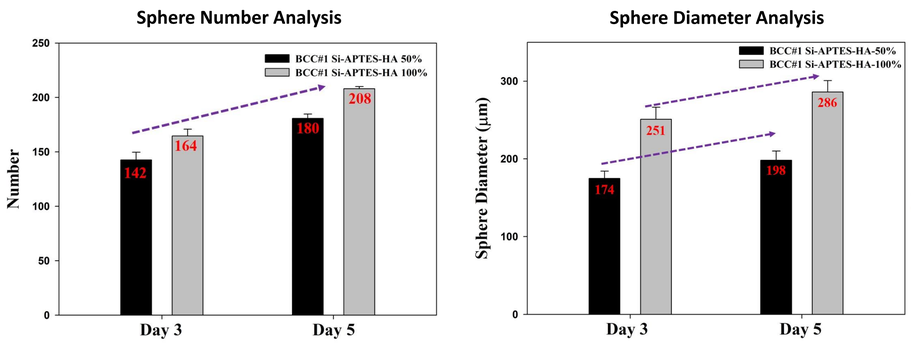


Fig 6. The number and size of HepG2 sphere in different culture conditions and culture days on BCCs-HA surface.

Cell Toxicity Test

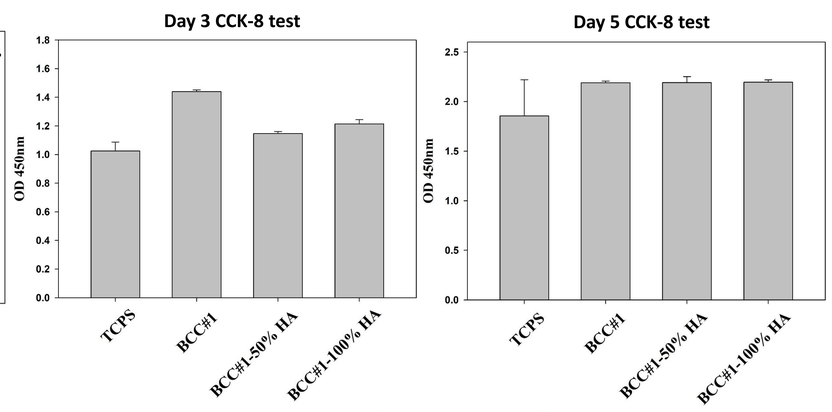


Fig 7. CCK-8 assay of HepG2 cell line on BCCs-HA surface material after day 3 and 5 of culture.

Analysis of CSCs Marker Expression

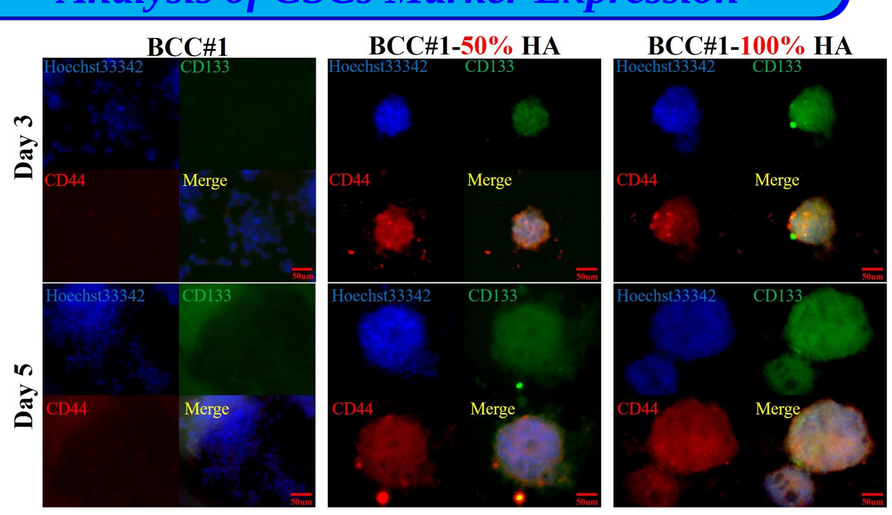


Fig 8. Microscopic images of immunofluorescence staining for CSC marker of CD133 (FITC), CD44 (PE) and nuclei (Hoechst 33342).

RT-PCR Analysis

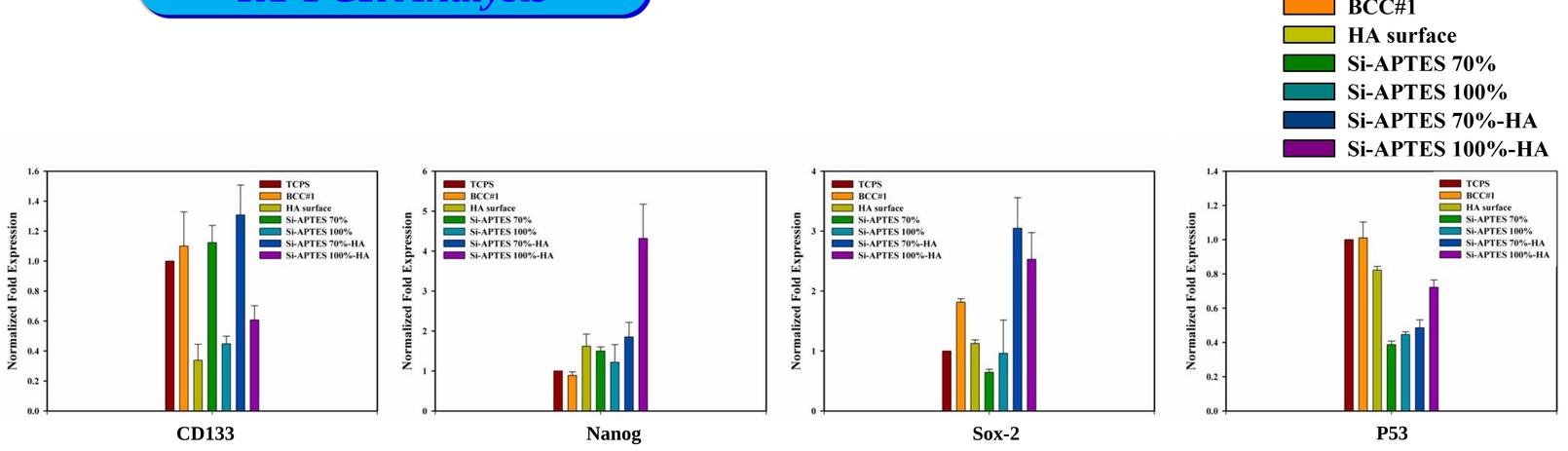


Fig 9. Expression of the CSC and stem cell gene CD133, Nanog, Sox-2 and The p53 tumour suppressor gene of HepG2 cells cultured on BCCs-HA culture system after 5 and days of culture.

Conclusion

The human hepatocellular cell lines (HepG2) was automatically aggregated and formed sphere on BCCs surface with different concentrations of HA. Mimicking Cancer stem cells (CSCs) niches in a defined manner could facilitate CSCs selection and production of the large number of CSCs. These results suggest that our culture systems may provide a label-free CSCs selection system on HepG2 cell line for future biomedical applications.

Reference

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